



PATENT
NY-HUBR 1230-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:)
Chris RUNDFELDT et al.)
) Group Art Unit: 1617
Application No.: 10/680,459)
)
Filed: October 6, 2003) Examiner: D.R. CLAYTOR
)
For: USE OF)
DIHYDROIMIDAZOLONES FOR) Confirmation No.: 4494
THE TREATMENT OF DOGS)
)
)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF PROFESSOR MARC VANDEVELDE
UNDER 37 C.F.R. § 1.132

I, Univ.- Prof. Dr. med. vet. Marc Vandeveld, Diplome of the European College of Veterinary Neurology (ECVN), do hereby make the following declaration:

1. I am an employee of the University of Bern (CH – 3001 Bern, Switzerland). I am head of the neurology section in the department of clinical veterinary medicine, at the Vetsuisse faculty of the University of Bern. A copy of my curriculum vitae is attached as Exhibit 1.

2. I have read and am familiar with the patent application referred to above, as well as the following publications: French, Am J Manag Care. 2001, 7(7 Suppl):S209-S214, Ross and Coleman, Neurosci. Behav. Rev. 24 (2000), 639-653, Berendt and Gram, J Vet Intern Med. 1999 13:14-20, Bialer et al., Epilepsy Research 43(2001), 11-58, and with the other references cited by the Examiner. I am also familiar with the body of literature dealing with epilepsy treatment in dogs and other pet animals.

3. I have read and am familiar with the arguments previously advanced by the applicants concerning the non-obviousness of the use of AWD 131-138 for the treatment of idiopathic epilepsy in dogs at the time the invention was made and the patent was filed, in view of the prior art cited by the Examiner.

In my opinion, the use of AWD 131-138 for the treatment of idiopathic epilepsy in dogs was not obvious at the time the subject patent application was filed.

4. One approach to the development of new antiepileptic drugs that is used frequently is the screening of new compounds in rodent seizure models and in animal models of epilepsy, such as the model of audiogenic seizures that require specific strains of subject rodents. Several such models have been used to characterize new compounds and to see whether these are potential antiepileptic agents in a target population, usually humans suffering from idiopathic epilepsy and idiopathic drug-resistant epilepsy in particular. For a review of antiepileptic discovery strategies please see Unverferth et al., Ullmann's Encyclopedia of Industrial Chemistry, 7th edition, Antiepileptics (a copy of which is attached).

5. AWD 131-138 has undergone such studies. The compound was tested in experimental animal models with electrical induction of seizures, chemical induction of seizures and in the audiogenic seizure model. The results of these tests have been published by Bialer et al., Epilepsy Research 43 (2001), 11-58, which is of record.

6. These models do not provide a suggestion that a compound would be useful as anticonvulsant for treatment of idiopathic epilepsy in dogs, as will now be shown.

7. In all models, the procedure used is the same: Adequate animals are selected and separated into a control group, at least one drug treatment group, or usually, several drug treatment groups. While the control group is exposed to a vehicle dose (administered orally or intraperitoneally with the drug carrier), the treatment groups receive the test drug in question and/or a reference drug at different dose levels, using

the same route of administration as the control. After a predefined time, based on kinetic data for the species, such as the time of maximal plasma or brain exposure after administration, seizures are induced. Such induction may be caused by injection of a convulsant drug such as pentylenetetrazole, by application of electrical shock or by other means such as by application of a very loud sound in models employing animals with audiogenic susceptibility for seizures. The response to this stimulus in treated groups of animals is compared to the response in the control group and the reference group.

8. As can be seen from this description, key to the testing of a candidate drug in such seizure models is the selection of the correct spacing between drug administration and seizure induction. Unfortunately, a human patient suffering from idiopathic epilepsy may experience seizures at any time. Therefore a compound for the successful treatment of idiopathic epilepsy in man needs to be active throughout a long period after administration. It is common knowledge that, after oral administration, drugs are absorbed, metabolized and finally excreted. This may take a few minutes, several hours, or even days. Only drugs which are present at therapeutically effective levels and which are active for at least several hours after dosing can be used for treatment of human patients with idiopathic epilepsy..

9. Drugs with rapid metabolism and rapid elimination are not successful anticonvulsants in man. Further more, they are not useful in dogs.

10. Drugs which are useful for treatment of human epilepsy are in most cases not useful for the treatment of canine epilepsy. This is clear from the literature dealing with treatment of epilepsy of dogs. For example in 1985 Löscher et al. analyzed data in order to validate the epileptic dog as a model for human epilepsy (Löscher et al., *Arzneimittelforschung* (Drug Research) 1985;35:82-87, (a copy of which is attached). They tested a number of commercial anticonvulsants used in humans in epileptic dogs. Contrary to their expectations the authors discovered that most available anticonvulsants, with the exception of phenobarbital and primidone, were not useful for

treatment of focal and generalized seizures in dogs suffering from idiopathic epilepsy. The authors concluded that these agents were not suitable for treating dogs because of their short half-lives. The authors list phenytoin, carbamazepine, valproic acid, diazepam, clonazepam, and nitrazepam as drugs which were not active and not suitable.

11. While phenytoin, carbamazepine and valproic acid are the mainstay of old generation antiepileptics for treatment of humans (see French, table 1), they were not suitable for use in dogs. All such compounds, however, are active in several animal models of seizures including those used to test AWD 131-138 (Rostock et al., *Epilepsy Research* 1996; 23:211-223, a copy of which is attached).

12. The latter reports demonstrate that data generated in experimental animal models are of limited relevance for the treatment of canine epilepsy. The data further show that the fact that a compound is a successful antiepileptic drug for treatment of human epilepsy does not predict clinically relevant activity in canine idiopathic epilepsy. Indeed, at the time of the invention set forth in the subject application, about 13 to 20 major antiepileptic drugs were on the market for the treatment of epilepsy in humans. French lists 13 compounds in table 1 (French, *Am J Manag Care*. 2001, 7(7 Suppl):S209-S214). In addition, clonazepam, diazepam, ethosuccimide and other compounds were also on the market. Bromides are used in some patients with drug resistant epilepsy, a number of minor drugs are available and further drugs were in the pipeline. None of these compounds are useful for treatment of canine epilepsy with the exception of phenobarbital and primidone and in some dogs also bromides. Phenobarbital and primidone remain the mainstays of canine anti-epileptic treatment.

13. As mentioned above, one possible reason why neither experimental animal data generated in seizure models nor data generated in human patients with idiopathic epilepsy can be extrapolated to epileptic dogs, is that canines metabolize chemicals very quickly, resulting in short half lives, and low plasma levels. The short half life of antiepileptic drugs in canines can not be compensated for with modified

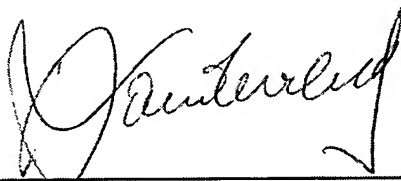
release formulations. Such formulations, which are well accepted and useful in man, can not be used in dogs. The dog, as a carnivore has a short gut and rapid gut passage is sufficient for canine digestion. Short intestinal passages are not compatible with modified release formulations which release active compound over several hours. Such formulations are often excreted in dogs without full release of active compound rendering their administration useless.

14. A further point must be considered related to the nature of the models used for testing AWD 131-138 (see Bialer et al. for effects of AWD 131-138 in these models). These models are models of induced seizures but not of epilepsy. In the maximal electroshock model, an otherwise healthy mouse or rat is exposed to an electrical shock to induce a generalized convulsion. In chemical seizure models, a compound capable of inducing convulsions is used to induce the seizures. In models employing audiogenic induction of seizures, mice with a genetic predisposition to induction of audiogenic seizures are exposed to high level sound which can induce convulsions, but only for a brief period during "adolescence" of the test animal. None of these animal models reflect true idiopathic epilepsy. Löscher et al. were aware of the lack of predictability of therapeutic usefulness of such animal models for treatment of idiopathic epilepsy in man. The latter authors therefore had considered using dogs suffering from idiopathic epilepsy, as a model for human idiopathic epilepsy; However, they failed to establish such animals as a model, This supports the position that canine idiopathic epilepsy is a complex phenomenon in a species who's biology and physiology differ vastly from humans and animal models of convulsion. Findings in respect to anti convulsive treatment in the latter, are therefore not applicable in dogs.

15. In conclusion, to one of ordinary skill in the art the data indicating that AWD 131-138 is effective in several animal models of seizures by no means suggest that AWD 131-138 is effective for the treatment of canine idiopathic epilepsy. Data generated in mouse models of audiogenic seizures do not change this view.

16. I finally declare that all statements made herein are based on my best knowledge and that I believe them to be true. Furthermore, I declare that these statements were made with the knowledge that intentionally false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated:

By: 

Professor Dr. Marc Vandeveld, Dipl. ECVN

Exhibit 1

Curriculum Vitae

Marc Vandeveld
Dr. med.vet., DECVN
Professor

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University of Bern, Switzerland
Bremgartenstrasse 109 a, 3012 Bern, Switzerland

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FAX: 0042 31 631 2538
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Born 24 June 1947 in Bruges, Belgium

- 1965-1971: Studies veterinary medicine, Univ. Gent, Belgium. Dr. med.vet.
1970-71: Student- assistant in dept. of vet. Physiology, Univ. Gent (prof G. Peeters).
Dissertation: „Milkejection in primiparous cows in the presence of their calves.“
1971: Training in human neurlogy/neuropathology Bunge institute (prof. L. van Bogaert),
Antwerp Belgium
1972-1974: Training in comparative neuropathology and veterinary neurology at the
institute of comparative neurology (prof. R. Fankhauser), University of Bern,
Switzerland.
1974-1979: Assistant professor at Scott-Ritchey foundation for small animal disease
research (prof. BF Hoerlein), Auburn university, US. Training in neurology and
exp. Pathology. Part-time in graduate programme at dept of comp. medicine,
Univ. of Alabama in Birmingham, training in biochemistry, cell biology and
laboratory animal medicine.
1979-1984: Assistant professor in institute of comparative neurology, Univ. of Bern,
Switzerland. Habilitation and appointment as Privatdozent for veterinary
neurology. Development of clinical neurology service. Neuropathological service.
Research.
1980 : Doctoral dissertation: „ Studies on chronic canine distemper virus
infection in the CNS“
1983 : Habilitation. Dissertation:“ Morphologic, immunocytochemical and immunological
studies on the pathogenesis of demyelination in canine distemper“
1984: Summer semester; Visiting professor, depts of neurology and pathology, univ. of
California in Davis
1985: Appointment as full professor and head of the institute of animal neurology,
University of Bern
1990 - : Responsible for the national Reference Laboratory for spongiform
encephalopathies. Since 1998: Reference lab for spongiform encephalopathies
of OIE.
1995: Diplomate (founding member) of the European college of Veterinary Neurology
2000 - 2005: Chairman of the department of clinical veterinary medicine, university of
Bern

Memberships

Swiss neuropathological society
World association for neuropathology
European Society for Veterinary Pathology
European Society for Veterinary Neurology(ex President)
European College of Veterinary Neurology (diplomate)
European Association for Veterinary Specialization (EAVS)

Review activity

Editorial board Acta Neuropathologica
Editorial board Vet J
Editorial board Ann. Med. Vet.
Editorial board Compendium der Veterinärmedizin
Referee for various research grants
Referee for various habilitations
Referee for various tenure procedures
Committee member and chairman for scientific evaluation of vet. faculties

Positions/committees

- Committee for cont. and postgrad. education, fac. vet med. Bern and Zürich 1989-95
- Evaluation committee of vet. faculty (chairman) 1990-1996
- Dean of the vet. faculty of Bern 1994 - 96
- Planning coordinator of vet. faculty 1996 -
- Member of the University senate 1997-
- President European Soc. Vet. Neurology 1987 - 90
- Chairman credentials committee, Europ. College Vet. Neurology 94-95
- Chairman of board of Ernest and Charlotte Frauchiger foundation
- Member of executive board of the European Association of Educational Veterinary Establishments (EAEVE) 1996-2000
- Committee for fusion of both swiss faculties 1997- 2000
- Chairman/member of various congress organisations

Research grants

• Swiss national science foundation:

CHF

1980-1981: Comparative Pathology of demyelinating diseases	187.000 -.
1982-1984: Pathogenesis of demyelination in canine distemper	246.197-.
1985-1987: Canine distemper virus-glial cell interaction in vitro	245.000 -.
1988-1991: Canine distemper-oligodendrocyte interaction	261.713 -.
1991-1992: Mechanism of virus induced cell damage in canine distemper virus infection	240.583 -.
1993-1996: Restricted Infection of oligodendrocytes	310.000 -.
1997-1999: Mechanisms of demyelination in canine distemper virus infection	162.000 -.
1997-1999: Mechanisms of distemper virus persistence (co-invest)	162.000 -.

• **Multiple Sclerosis Society**

1991	Is demyelination in canine distemper the result of oligodendroglial infection?	25'000.-.
1992	Mechanisms of viral persistence in canine distemper (co-invest)	30'000.-.
1993	Studien zur abortiven Infektion in Oligodendrozyten (co-invest)	30'000.-.
1994	Is the canine distemper virus leader responsible for restricted infection in oligodendrocytes? (co-invest)	30'000.-.
1995	Mechanisms of viral persistence in the CNS (co-invest).	25'000.-.
1997	Mechanisms of distemper virus induced degeneration of Oligodendrocytes.	20.000 -
1999:	Degeneration of oligodendrocytes	30.000.-.

• **Swiss Veterinary office;**

1992	Detection of spongiform encephalopathies in Switzerland	120'000.-.
1993	Immunological and molecular biological techniques for detection of TSE in Switzerland	120'000.-.
1994	Immunological and molecular biological techniques for detection of TSE in Switzerland	120'000.-.
1996-1999:	National reference lab for spongiform encephalopathies and neurocenter (in collaboration with nat. inst. for virology and immunoprophylaxis). Studies on true incidence of TSE in Switzerland. Development and validation of test systems. Epidemiological studies.	1.200'000.-.

• **Others**

1990-93	In vivo ELISA test of CDV in the CSF. MERIEUX Co, France.	100,000.-
1998-2001	FAIR Programme (EU); Partner im Projekt über Molecular hereditary factors in spongiform encephalopathies (MASSES). (subcontractor): PrP expression in Bovines	425.000 -

Awards

- Honorary member of the Italian society for veterinary science.
- WSAVA international award for scientific achievement 2004

Professional activity

• Teaching:

- Undergraduate: Neurology course, Neuropathology (in course: Pathology of organsystems)
- Post graduate:
 - * Clinical neurology: Untill 1990 training of veterinarians (total of 5 Residents). Untill 1992 active in national and international continuing education.
 - * Neuropathology: weekly slide seminars. Neuropathology courses for pathologists (ca 1 week/year)

• Research:

Predominantly in the field of Neuropathology/Neuroimmunology/Neurovirology. Special interest: pathogenesis of demyelination in distemper. Collaboration in various other clinical and pathological research projects.

• Service:

- Clinics: 1980 – 1991: Development of clinical neurology service and residency programme in collaboration with clinics of the vet school.
- Neuropathology: 1980 - Regular diagnostic activity. Since 1990 responsible for national reference lab for spongiform encephalopathies (since 1998 also reference lab for OIE)

• Administration/management :

- Director of institute of animal neurology for 15 years (ca. 20 collaborators)
- Faculty administration (Dean for 2 years, coordinator of planning for 9 years, other committees)
- Chairman of department of clinical veterinary medicine (includes all clinics of the vet school, ca.100 academic, 50 support staff) for 5 years

• Career development

- Training of Specialists in veterinary neurology: (clinical neurology, bis 1990): P. Bichsel, M. Cachin, M. Kornberg, A. Jaggy, A. Tipold, M. Wolf
- Training in research; Doctoral students /Postdocs: A. Glaus, B. Dubacher, C. Botteron, C. Griot, M. Griot-Wenk, A. Zurbriggen, M. Kornberg, M. Brügger, D. Hamburger, S. Cerutti, E. Bollo
- Habilitations: A. Zurbriggen, A. Tipold, A. Jaggy

Languages

Fluent (speaking, writing) German, French, Dutch, English

Publications

Vandeveld M., Fankhauser R.: Zur Pathologie der Rückenmarksblutungen beim Hund. Schweiz.Arch.Tierheilk. 114, 463-475 (1972)

Peeters G., Debuysscher E., Vandeveld M.: Milk Ejection in Primiparous Heifers in the Presence of Their Calves. Zblt. Vet.Med. 20a, 7, 531-536 (1973)

Fankhauser R., Freudiger U., Vandeveld M., Fatzer R.: Purkinjezellatrophie nach Masernvirus-Vakzinierung beim Hund. Schweiz.Arch.Tierheilk. 112, 353363 (1973)

Vandeveld M.: Epilepsie und Choreo-Athetose bei einem Husarenaffen (*Erythrocebus patas*) mit diffuser Erkrankung der Stammganglien. Schweiz.Arch. Tierheilk. 115, 465-474 (1973)

Ueltschi G., Steinberg S.A., Vandeveld M.: Darstellung einer Kleinhirnaplasie mit Hydrozephalie beim Kalb mittels Pneumoenzephalographie. Schweiz. Arch.Tierheilk. 115, 471-474 (1973)

Vandeveld M., Fatzer R.: Neurologische Komplikaties bij drie Honden na Vaccinatie met een Rabies weefselkultuurvaccin. VI. Diergeneesk.Tsch. 43, 253-259 (1974)

Vandeveld M., Fankhauser R.: A Leucodystrophy in Miniature Rabbits. Fol. vet. lat. 3, 620 (1973)

Häni H., Vandeveld M., Scholl E.: Enzootisches Auftreten von Maulbeerherzkrankheit beim Schwein. Schweiz.Arch.Tierheilk. 116, 295-301 (1974)

Steck F., Scholl E., Vandeveld M., Häni H., Hartmann H., Kilchsperger G., Pohlenz G.J.: Zum Vorkommen des Morbus Aujeszky beim Schwein in der Schweiz. Schweiz.Arch.Tierheilk. 116, 7 (1974)

Vandeveld M., Müller R.: Syringomyelie bei einem Schwarz-Panther. Acta Zoologica et Pathologica Antwerpiensa,58, 123-130 (1974)

Vandeveld M., Fatzer R., Fankhauser R.: Chronisch progressive Formen der Staupe-Enzephalitis des Hundes. Schweiz.Arch.Tierheilk. 116, 391404 (1974)

Steinberg A., Vandeveld M.: A comparative study of two methods of cytological evaluation of spinal fluid in domestic animals. Folia vet.lat. 4, 235 (1974)

Steck F., Schären B., Fatzer R., Vandeveld M., Scholl E., Häni H.: Vomiting and wasting disease bei Ferkeln in der Schweiz. Schweiz.Arch. Tierheilk. 117, 617-622 (1975)

Vandeveld M., Higgins R. J., Greene C. E.: Neoplasms of mesenchymal origin in the spinal cord and nerve roots of three dogs. Vet.Path. 13, 47-48 (1976)

Greene C.E., Vandeveld M., Braund K.G.: Lissencephaly in two Lhasa Apso dogs. JAVMA 169, 405-410 (1976)

Vandeveld M., Greene C.E., Hoff E.J.: Lower motor neuron disease with accumulation of neurofilaments in a cat. Vet.Path. 13, 428-435 (1976)

Vandeveld M., Fatzer R., Fankhauser R.: Atypical lesions of the CNS associated with canine distemper. Proc. VIIIth Int.Congr. Neuropathology. Excerpt.Med. Amsterdam, 513-516 (1975)

Vandeveld M., Braund K.G., Hoff J.E.: Central neurofibromas in the dog: A study of two cases. Vet.Pat. 14, 470-478 (1977)

Taylor H.W., Vandeveld M., Firth E.: Spinal cord infarction due to fibrocartilagenous emboli in the horse. Vet.Path. 14, 479-481 (1977)

Greene C.E., Vandeveld M., Hoff E.J.: Cerebrospinal hypomyelinogenesis in a pup. JAVMA 171, 534-536 (1977)

Higgins R.J., Vandeveld M., Braund K.G.: Internal hydrocephalus in young dogs associated with periventricular encephalitis. Vet.Path. 14, 236-246 (1977)

Higgins R.J., Vandeveld M., Hoff E.J., Jagar J.E., Cork L.K., Silberman M.S.: Neurofibrillary accumulation in the zebra (*Equus burchelli*). Acta Neuropathol.(Berl.) 37, 1-5 (1977)

Braund K.G., Vandeveld M., Albert R.A., Higgins R.J.: Central (postretinal) visual impairment in the dog. A clinical-pathological study. J.Small Anim.Pract. 18, 395-405 (1977)

Swaim S.T., Vandeveld M.: Clinical and histological evaluation of bilateral and deep dorsal hemilaminectomy in the dog. JAVMA 170, 407-413 (1977)

Vandeveld M., Spano J.S.: Cerebrospinal fluid cytology in canine neurological disease. Am. J. Vet. Res. 38, 1827-1832 (1977)

Vandeveld M., Boring G.B., Hoff E.J., Gingerich D.A.: the effect of Levamisole on the canine central nervous system. J.Neuropath.Exp.Neurol. 37, 165-173 (1978)

- Vandeveld M., Kristensen B.: Observations on the distribution of canine distemper virus in the central nervous system in dogs with demyelinating encephalitis. *Acta Neuropathol.(Berl.)* 40, 233-236 (1977)
- Hoerlein B.F., Vandeveld M.: Primary diseases of the brain and spinal cord. In *Canine Neurology. Diagnosis and Treatment*. Hoerlein 3rd ed. Lea and Febiger, 1977
- Kristensen B., Vandeveld M.: Immunofluorescence studies of canine distemper encephalitis on paraffin embedded tissue. *Am.J.Vet.Res.*, 1017-1021 (1978)
- Braund K.G., Vandeveld M.: German shepherd myelopathy: A morphologic and morphometric study. *Am.J.Vet.Res.* 39, 1309-1315 (1978)
- Vandeveld M., Kristensen B., Greene C.E.: Primary reticulosis in the canine brain. *Vet.Path.* 15, 673-675 (1978)
- Nettles V.F., Vandeveld M.: Thalamic ependymoma in the whitetailed deer. *Vet.Path.* 15, 133-135 (1978)
- Braund K.G., Vandeveld M., Walker J.L., Redding R.W.: Disseminated granulomatous encephalitis in six dogs. *JAVMA* 172, 1195-1200 (1978)
- Vandeveld M., Braund K.G., Walker T.L., Kornegay: Dysmyelination in the Chow Chow dog. *Acta Neuropathol.(Berl.)* 42, 211-215 (1978)
- Schields R., Vandeveld M.: A spontaneous lower motor neuron disease in the rabbit. *Acta Neuropathol.(Berl.)* 44, 85-90 (1978)
- Swaim S.F., Vandeveld M., Faircloth J.C.: Evaluation of brain biopsy techniques in the dog. *JAAHA* 15, 627-633 (1979)
- Greene C.E., Vandeveld M.: Lissencephaly. In: *Spontaneous Animal Models for Human Disease*. Andrews, Altman, Ward eds. Academic Press, New York, (1979)
- Braund K. G., Walker T. L., Vandeveld M. : Fascicular nervebiopsy in the dog. *Am.J.Vet.Res.* 40, 1025-1030 (1979)
- Braund K.G., Vandeveld M. : Polioencephalomalacia in 25 dogs. *Vet.Path.* 16, 661-672 (1979)
- Lisiak J. A. , Vandeveld M.: Studies on polioencephalomalacia associated with canine distemper virus infection. *Vet.Path.* 16, 650-660 (1979)

- Stowe H. D. , Vandeveld M.: Lead encephalopathy in dogs fed high fat low calcium diets . J . Neuropath . Exp . Neurol . 38, 463-474 (1979)
- Vandeveld M., Braund K. G . : Polioencephalomyelitis in cats . Vet . Path . 16, 420-427 (1979)
- Fankhauser R., Vandeveld M. : Liquoruntersuchung. Kleintierpraxis 24, 205-252 (1979)
- Vandeveld M.: Degenerative disorders of the spinal cord . Vet . Clin . North Am. 10, 147-154 (1980)
- Vandeveld M., Schawalder P., Ueltschi G.: Degenerative Myelopathie beim Deutschen Schäfer. Schweiz.Arch.Tierheilk. 122, 323-326 (1980)
- Luttgen P.J., Braund K.G., Brawner W.R., Vandeveld M. : Retrospective study of twenty-nine spinal tumors in the dog and cat. J.Small Anim.Pract. 21, 213-226 (1980)
- Vandeveld M., Fatzer R.: Neuronal ceroid-lipofiscinosis in older dachshounds. Vet.Path. 17, 686-692 (1980)
- Vandeveld M.: Primary reticulosis of the central nervous system. Vet. Clin. North Am. 1 O, 57-63 (1980)
- Vandeveld M., Kristensen B., Braund K. G., Greene C . E . , Swango L . S . , Hoerlein B.F.: Chronic canine distemper encephalitis. Vet.Path. 17, 17-29 (1980)
- Swaim F.S., Vandeveld M., Sommers W.C., Baine L., McGuire J.A. : Comparison of hypothermic and normothermic spinal cord perfusion in the dog. Vet. Surg. 8, 119-124 (1979)
- Vandeveld M., Meier C.: Multiple sclerosis and canine distemper encephalitis: An epidemiological approach. J.Neurol.Sci. 47, 255-260 (1980)
- Vandeveld M., Fankhauser R. , Kristensen F., Kristensen B. : Immunoglobulins in demyelinating lesions in canine distemper encephalitis: An immunohistological study. Acta Neuropathol. (Berl.) 54, 31-41 (1981)
- Vandeveld M., Fatzer R., Fankhauser R.: Immunohistological studies on primary reticulosis in the canine brain. Vet.Path. 18, 577-588 (1981)
- Hoff E.J., Vandeveld M. : Non suppurative encephalomyelitis in cats. Suggestive of a viral origin. Vet.Path. 18, 170-180 (1981)

Vandeveld M., Braund K. G., Luttgen P. J., Higgins R. J. : Dysmyelination in Chow Chow dogs; further studies in older dogs. *Acta Neuropathol.(Berl.)* 55, 81 -87 (1981)

Wouda W., Vandeveld M. : Internal hydrocephalus of presumably postinfectious origin in young dogs. *Zbl.Vet.med.A* 28, 481-493 (1981)

Hoff E. J., Vandeveld M. : Necrotizing vasculitis in the central nervous system of two dogs. *Vet.Path.* 18, 219-223 (1981)

Vandeveld M., Oettli P., Fatzer R., Rohr M.: Polyradiculoneuritis beim Hund; klinische, histologische und elektronenmikroskopische Befunde. *Schweiz. Arch.Tierheilk.* 123, 207-217 (1981)

Fankhauser R., Vandeveld M. : Zur Klinik der Tumoren des Nervensystems bei Hund und Katze. *Schweiz.Arch.Tierheilk.* 123, 553-571 (1981)

Vandeveld M.: Spinal cord compression. In *Pathophysiology in Small Animal Surgery*. M. J. Bojrab ed. Lea & Febiger, 1981

Bichsel P., Vandeveld M. : Un cas de lipofuscinose céroïde chez un berger de Yougoslavie. *Schweiz. Arch. Tierheilk.* 124, 413-418 (1982)

Fankhauser R., Vandeveld M., Zwahlen R. : Scrapie in der Schweiz? *Schweiz. Arch. Tierheilk.* 124, 227- 232 (1982)

Kristensen B., Kristensen F., Vandeveld M., Higgins R.J., de Weck A.L. : Canine lymphocyte cultures in vitro: Evaluation of peripheral blood lymphocyte response to mitogens. *Vet.Immunol.Immunopath.* 3, 439-448 (1982)

Kristensen F., Kristensen B., Vandeveld M., Higgins R.J., de Weck A.L. : Analysis of the in vitro activation and proliferation process in lymphocytes derived from canine thymus and mesenteric lymphnode. *Vet.Immunol. Immunopath.* 2, 579-590 (1981)

Vandeveld M., Kristensen F., Kristensen B., Steck A.J., Kihm U. : Immunological and pathological findings in demyelinating encephalitis associated with canine distemper infection. *Acta Neuropathol. (Berl.)* 56, 1-8 (1982)

Steck A.J., Vandeveld M.: Immunological studies in demyelinating encephalitis associated with vaccinia virus and canine distemper virus infection. *Progress Brain Res.*, Behan, terMeulen, Rose eds. 59, 275-297 (1983)

Vandeveld M, Fankhauser R, Luginbuhl H: Comparative aspects of gliomas in animals. *Proc. IV neurosurgical Congress (Mainz)* 1983

Vandeveld M., Higgins R. J., Kristensen F., Kristensen B., Steck A. J., Kihm U.:
Demyelination in experimental canine distemper encephalitis; immunological,
pathological and immunopathological studies. *Acta Neuropathol. (Berl.)* 56, 285-293
(1982)

Vandeveld M., Fankhauser R., Wiesmann U., Herschkowitz N. : Hereditary
neurovisceral mannosidosis associated with alpha-mannosidase deficiency in Persian
cats . *Acta Neuropathol . (Berl)* 58, 64-68 (1982)

Vandeveld M., Hugli E., Isler A., Suter M., Pfister H.: Histological immunoenzyme
techniques: Evaluation of various methods and modifications. *Res.Vet. Sci.* 34, 193-198
(1983)

Cerruti-Sola S., Kristensen F., Vandeveld M., Bichsel P., Kihm U.: Lymphocyte
responsiveness to lectin and myelin antigens in canine distemper infection in relation to
the development of demyelinating lesions. *J. Neuroimmunol.* 4, 77-90 (1983)

Kristensen F., Walker C., Kristensen B., Vandeveld M., de Weck A.L.: Technical
aspects of low ³H-thymidine incorporation by mitogen stimulated canine peripheral
blood lymphocytes in vitro. *Vet.Immunol.Immunopathol.* 3, 557-566 (1982)

Fankhauser R., Vandeveld M.: Leucodystrophies in animals. *Bull.Soc. Belge Ophthal .*
208 I, 399-404 (1983)

Vandeveld M., Fankhauser R.: Neuroimmunologische Krankheiten bei Haustieren.
Dtsch.tierärztl.Wschr. 90, 310-313 (1983)

von Beust B.R., Vandeveld M., Tontis A., Spichtig M.: Enzootische Ataxie beim
Zicklein in der Schweiz. *Schweiz.Arch.Tierheilk.* 125, 345-351 (1983)

Bichsel P., Vandeveld M., Lang J.: Degenerative Myelopathie bei grossen
Hunderassen. *Tierärztl.Prax.* 12, 239-240 (1984)

Wouda W., Vandeveld M., Oettli P., Hoerlein B.F.: Sensory neuropathy in dogs: a
study of four cases. *J.Comp.Path.* 93, 437-450 (1983)

Bichsel P., Vandeveld M., Lang J.: L'infarctus de la moelle epiniere à la suite
d'embolies fibrocartilagineuses chez le chien et le chat. *Schweiz.Arch.Tierheilk.* 126,
387-397 (1984)

Bichsel P., Vandeveld M., Lang J.: Degenerative myelopathy in a family of Husky
dogs. *JAVMA* 183, 998-1000 (1983)

Steck A.J., Meier C., Vandeveld M., Regli F.: Polyneuropathies et gammopathies: une
forme avec anticorps antiglycoprotéine MAG. *Rev.Neurol.* 140, 28-36 (1984)

Vandeveld M., Bichsel P., Cerruti-Sola S., Steck A., Kristensen F., Higgins R.J.: Glial proteins in canine distemper virus-induced demyelination. *Acta Neuropathol.(Berl.)* 59, 269-276 (1983)

Zurbriggen A., Vandeveld M.: Canine distemper virus-induced glial cell changes in vitro. *Acta Neuropathol.(Berl.)* 62, 51-58 (1983)

Zurbriggen A., Vandeveld M., Beranek C.F., Steck A.: Morphological and immunocytochemical characterization of mixed glial cell cultures derived from neonatal canine brain. *Res.Vet.Sci.* 36, 270-275 (1984)

Cerruti-Sola S., Kristensen F., Vandeveld M., de Weck A.L.: Interleukin 1- and 2-like activities in the dog. *Vet.Immunol.Immunopathol.* 6, 261 (1984)

Steck A.J., Murray N., Vandeveld M., Zurbriggen A.: Human monoclonal antibodies in demyelinating neuropathy are specific for myelin associated glycoprotein. *J.Neuroimmunol.* 5, 145-156 (1983)

Meier C., Steck A., Vandeveld M.: Immunopathogenese der Polyneuropathien bei Paraproteinämie. *Dtsch.med.Wschr.* 109, 828-833 (1984)

Meier C., Vandeveld M., Steck A., Zurbriggen A.: Demyelinating polyneuropathy associated with monoclonal IgM-paraproteinaemia. Histological, ultrastructural and immunocytochemical studies. *J.Neurol.Sci.* 63, 353-367 (1983)

Bichsel P., Vandeveld M., Vandeveld E., Affolter U.: Immuno-electrophoretic determination of albumin and IgG in serum and cerebro spinal fluid in dogs with neurological diseases. *Res.Vet. Sci.* 37, 101-107 (1984)

Zurbriggen A., Vandeveld M., Steck A., Angst B.: Myelin associated glycoprotein is produced before myelin basic protein in cultured oligodendrocytes. *J.Neuroimmunol.* 6, 41-49 (1984)

Vandeveld M., Fankhauser R., Luginbühl H.: Immunocytochemical studies in canine neuroectodermal brain tumors. *Acta Neuropathol.(Berl.)* 66, 111-116 (1985)

Dumas M., Zurbriggen A., Vandeveld M., Hye Yim S., Arnason B.G.W., Szuchet S., Meier C.: A monoclonal antibody that binds to both astrocytes and myelin sheaths. *J.Neuroimmunol.* 9, 55-67 (1985)

Vandeveld M.: Comparative aspects of neurospinal dysraphism in domestic animals
Proc . V . Neurosurg . Congress Mainz . 1985

Vandeveld M., Zurbriggen A., Dumas M., Palmer D. : Canine distemper virus does not infect oligodendrocytes in vitro. J.Neurol.Sci. 69, 133-137 (1985)

Vandeveld M., Zurbriggen A., Higgins R.J., Palmer D. : Spread and distribution of viral antigen in nervous canine distemper. Acta Neuropathol.(Berl.) 67, 211 -218 (1985)

Bichsel P., Lang J., Vandeveld M., Haeni H.J., Oettli P.: Solitary cartilaginous exostoses associated with spinal cord compression in three largebreed dogs . J . Amer . Anim . Hosp . Ass . 21, 619- 622(1985)

Vandeveld M., Zurbriggen A., Steck A., Bichsel P. : Studies on the intrathecal humoral immune response in canine distemper encephalitis. J. Neuroimmunol. 11 41-51 (1986)

Zurbriggen A., Vandeveld M., Dumas M.: Secondary degeneration of oligodendrocytes in canine distemper virus infection in vitro. Lab.Investigation. 54, 424-431 (1986)

Bollo E., Zurbriggen A., Vandeveld M., Fankhauser R.: Canine distemper virus clearance in chronic inflammatory demyelination. Acta Neuropathol. (Berl) 72, 69-73 (1986)

Zurbriggen A., Vandeveld M., Bollo E.: Demyelinating, non-demyelinating and attenuated canine distemper virus strains induce oligodendroglial cytolysis in vitro . J . Neurol . Sci ., 79, 33-41 (1987)

Vandeveld M., Fankhauser R.: Einführung in die veterinärmedizinische Neurologie. Pareys Studentexte. Verlag Paul Parey, Berlin und Hamburg, 1987

Zurbriggen A., Dumas M., Vandeveld M.: Neurons in dissociated canine brain cell cultures. J.Vet.Med. A 34, 673-678 (1987)

Zurbriggen A., Vandeveld M., Dumas M., Griot C., Bollo E.: Oligodendroglial pathology in canine distemper virus infection in vitro. Acta Neuropathol.(Berl.) 74, 366-373 (1987)

Vandeveld M., Richard A., Fankhauser R.: Liquoruntersuchungen bei neurologisch kranken Hunden und Katzen. Schweiz.Arch.Tierheilk. 129,443-456 (1987)

Lang J., Huber P., Vandeveld M.: Erfahrungen mit der Computertomographie in der Kleintierneurologie. Schweiz.Arch.Tierheilk. 130, 167-183 (1988)

Jaggy A., Gaillard C., Lang J., Vandeveld M.: Hereditary cervical spondylopathy (Wobbler Syndrome) in the Barzoi dog. J. Am. animal Hosp. Assoc., 453 - 460 (1987)

Jaggy A., Vandeveld M.: Multisystem neuronal degeneration in Cocker Spaniels, J. Vet. Internal Med. 2 : 117 - 120 (1988)

Regnier A., Vandeveld M., Thevenet A.: Dysautonomie feline (syndrome de Key-Gaskell): Observation d'un cas en France et revue de la litterature. *Revue Med.Vet.* 138, 793-797 (1987)

Griot C., Vandeveld M.: Transferrin, carbonic anhydrase C and ferritin in dissociated murine brain cell cultures. *J. Neuroimmunol.* 18 : 333 - 340 (1988)

Mutinelli F., Vandeveld M., Griot C., Richard A.: Astrocytic infection in canine distemper virus-induced demyelination, *Acta Neuropathol. (Berl.)* 77 : 333 - 335 (1988)

Fankhauser R., Vandeveld M.: Veterinary neurology - past, present and future, *J. Comp. Path.* 98 : 275 - 286 (1988)

Griot C., Bürge T., Brigger S., Richard A., Peterhans E., Vandeveld M.: Makrophagen bei der zentralnervösen Hundestaupe: Freunde oder Feinde? *Schweiz. Arch. Tierheilk.* 131:351-359 (1989)

Leupold U., Martig J., Vandeveld M. : Diagnostische Aspekte neurologischer Krankheiten des Rindes. Eine retrospektive Studie. *Schweiz. Arch. Tierheilk.* 131:327-340 (1989)

Griot C., Bürge T., Vandeveld M., Peterhans E.: Antibody-induced generation of reactive oxygen radicals by brain macrophages in canine distemper encephalitis: a mechanism for bystander demyelination. *Acta Neuropathol. (Berl.)* 78:396-403 (1989)

Bürge T., Griot C., Vandeveld M., Peterhans E.: Antiviral antibodies stimulate production of reactive oxygen species in cultured canine brain cells infected with canine distemper virus. *J. Virol.* 63:2790-2797 (1989)

T. Glaus, C. Griot, A. Richard, U. Althaus, N. Herschkowitz, M. Vandeveld: Ultrastructural and biochemical findings in brain cell cultures infected with canine distemper virus. *Acta Neuropathol.* 80: 59- 67(1990)

C. Griot, M. Vandeveld, A. Richard, E. Peterhans, R. Stocker. Selective degeneration of oligodendrocytes mediated by reactive oxygen species. *Free Rad. Res. Comms* 11: 181- 193(1990)

M. Wolf, M. Cachin, M. Vandeveld, Andrea Tipold, J.P. Dubey. Zur klinischen Diagnostik des protozoären Myositissyndroms (*Neospora Caninum*) des Welpen. *Tierärztl. Prax.* 19: 302- 306(1991)

M. Cachin, M. Vandeveld, A. Zurbriggen. Ein Fall von spongiformer Enzephalopathie ("Rinderwahnsinn") bei einer Kuh in der Schweiz. *Schweiz. Arch. Tierheilk.* 133: 53- 57(1991)

M. Cachin, M. Vandevelde. Congenital tremor with spongy degeneration of the central nervous system in two puppies. *J. Int. Vet. Med.* 5: 87- 90(1991)

Cachin M, Vandevelde M: Tremor beim Tier. *Wien. Tierärztl. Mschr.* 78: 252-255, 1991

Vandevelde M, Zurbriggen A, Fatzer R. 1992. Die Spongiforme encephalopathien mit besonderer berücksichtigung der bovinen spongiformen Enzephalopathie. *Schweiz med Wschr.* 122: 887-892.

Vandevelde M, Cachin M.1992 The neurologic form of canine distemper. In *Current Veterinary therapy* . Kirk ed. 1003-1007.

Tipold A, Vandevelde M, Jaggy A. 1992. Neurological manifestations of canine distemper virus infection. *J. small anim. pract.* 33: 466-470

P.M. Moreau, J.M. Vallat, J. Hugon, M.J. Leboudet, M. Vandevelde. Peripheral and central distal axonopathy of suspected inherited origin in Birman cats. *Acta Neuropathol.*82: 143- 146(1991)

M. Griot- Wenk, C. Griot, H. Pfister, M. Vandevelde. Antibody-dependent cellular cytotoxicity in antimyelin antibody induced oligodendrocyte damage in vitro. *J. Neuroimmunol.* 33: 145- 155(1991)

D. Hamburger, C. Griot, A. Zurbriggen, C. Oervell, M. Vandevelde. Loss of virulence of canine distemper virus is associated with a structural change recognized by a monoclonal antibody. *Experientia.* 47: 842- 845(1991)

C. Botteron, A. Zurbriggen , C. Griot, M. Vandevelde. Canine distemper virus- immune complexes induce bystander degeneration of oligodendrocytes. *Acta Neuropathol.* 83, 402-407 (1992)

Brügger, M., T. W. Jungi, A. Zurbriggen, and M. Vandevelde. 1992. Canine distemper virus increases procoagulant activity of macrophages. *Virology* 190:616-623.

Tipold, A., H. Pfister, and M. Vandevelde. 1993. Determination of the IgG index for the detection of intrathecal immunoglobulin synthesis in dogs using an ELISA. *Res. Vet. Sci.* 54:40-44.

Yamawaki, M., A. Zurbriggen, A. Richard, and M. Vandevelde. 1993. Saponin treatment for in situ hybridization maintains good morphological preservation. *J. Histochem. Cytochem.* 41:105-109.

Zurbriggen, A., C. Müller, and M. Vandevelde. 1993. In situ hybridization of virulent canine distemper virus in brain tissue, using digoxigenin-labeled probes. *Am. J. Vet. Res.* 54:1457-1461.

Zurbriggen, A., M. Yamawaki, and M. Vandevelde. 1993. Restricted canine distemper virus infection of oligodendrocytes. *Lab. Invest.* 68:277-284.

Graber H, Zurbriggen A., Vandevelde M.: Identification of canine glial cells by nonradioactive in situ hybridisation. *J.Vet.Med.A* 40: 665-671, 1993

Cachin M., Vandevelde M.: Neurologische Erkrankungen. in: Kompendium der Heimtierkrankheiten 2. E. Wiesner ed. Gustav Fischer, Stuttgart. 189-211, 1993.

Vandevelde M., Wolf M.: Spinal cord compression in: Disease mechanisms in small animal surgery. Bojrab ed. Lea and Febiger, Philadelphia. 1152-1157, 1993

Fankhauser R., Vandevelde M.: Zentrales und peripheres Nervensystem in: Klinik der Hundekrankheiten. Freudiger, Grünbaum, Schimke eds. Gustav Fischer Stuttgart. 679-713, 1993.

M. Wolf, M. Cachin, M. Vandevelde, Andrea Tipold, J.P. Dubey. Zur klinischen Diagnostik des protozoären Myositissyndroms (*Neospora Caninum*) des Welpen. *Tierärztl. Prax.* 19: 302- 306(1991)

M. Cachin, M. Vandevelde, A. Zurbriggen. Ein Fall von spongiformer Enzephalopathie ("Rinderwahnsinn") bei einer Kuh in der Schweiz. *Schweiz. Arch. Tierheilk.* 133: 53-57(1991)

M. Cachin, M. Vandevelde. Congenital tremor with spongy degeneration of the central nervous system in two puppies. *J. Int. Vet. Med.* 5: 87- 90(1991)

Tipold A, Vandevelde M, Jaggy A. 1992. Neurological manifestations of canine distemper virus infection. *J. small anim. pract.* 33: 466-470

Tipold A., Pfister H., Zurbriggen A., Vandevelde M.: Intrathecal synthesis of major immunoglobulin classes in inflammatory diseases of the canine CNS. *Vet Immunol. Immunopathol.* 42:149-159, 1994

M. Brügger, E. Dayrell-Hart, C. Meier, M. Vandevelde. A chemoluminescence assay to detect antibodies to brain surface antigens in human sera. *J. Neuroimmunol.* 49: 215-219, 1994

Tipold A., Jaggy A., Zurbriggen A., Vandevelde M.: Neurologische symptome bei Hundestaupe-eine klinische Studie. *Wien. Tierärztl.Mschr.* 81:274-279, 1994.

Zurbriggen A., Vandevelde M.: The pathogenesis of nervous distemper. *Progr. vet Neurol.* 5: 109-116, 1994.

Vandeveld M., Zurbriggen A.: The neurobiology of canine distemper virus infection. Vet.microbiol. 44: 271-280, 1995

Tipold A., Vandeveld M., Zurbriggen A.: Neuroimmunological studies in steroid-responsive meningitis-arteritis in dogs. Res.vet.Sci 58: 103-108, 1995.

Zurbriggen A., Graber H., Wagner A., Vandeveld M.: Canine distemper persistence in the nervous system is associated with noncytolytic virus spread. J.Virol. 69: 1678-1686, 1995.

Müller C., Fatzer R., Beck K., Vandeveld M., Zurbriggen A.: Studies on canine distemper virus persistence in the nervous system. Acta Neuropathol. 89: 438-445, 1995.

Zurbriggen A., Graber H., Vandeveld M.: Selective spread and reduced viral release leads to canine distemper virus persistence in the nervous system. Vet. Microbiol. 44: 281-288, 1995

Graber H., Meyer R., Fatzer R., Vandeveld M., Zurbriggen A.: In Situ Hybridisation and immunocytochemistry for Prion protein (PrP) in bovine spongiform encephalopathy (BSE). J. Vet. Med. A. 42, 454-459, 1995

Graber H., Müller C., Vandeveld M., Zurbriggen A.: Restricted infection with canine distemper virus leads to down-regulation of myelin gene transcription in cultured oligodendrocytes. Acta Neuropathol 90: 312-318, 1995

Fatzer R., Graber H., Meyer R., Cardozo C., Vandeveld M., Zurbriggen A.: Neuronal degeneration in brainstem nuclei in bovine spongiform encephalopathy. J. Vet. Med. 43: 23-29, 1996

Stettler M, Beck K, Wagner A, Vandeveld M and Zurbriggen A: Determinants of persistence in canine distemper viruses. Veterinary Microbiology. 57:83-89, 1997.

Zurbriggen A, Schmid I, Graber HU, Vandeveld M: Oligodendroglial pathology in canine distemper. Acta Neuropathol (Berl). 95: 71-77, 1997

Jungi TW, Adler H, Adler B, Fatzer R, Werner ER, Zurbriggen A, Vandeveld M and Peterhans E: Inducible nitric oxide synthase expression by goat macrophages in vivo and in vitro. A comparison with cattle macrophages. Handbook of Sheep and Goat Immunology. Academic Press. 1997

Jungi TW, Pfister H, Sager H, Fatzer R, Vandeveld M and Zurbriggen A: comparison of inducible nitric oxide synthase expression in the brains of Listeria monocytogenes-infected cattle, sheep and goats and in macrophages stimulated in vitro. Infection and Immunity, 65: 5279-5288, 1997.

Heim D, Fatzer R, Hörnlmann B, Vandevelde M: Häufigkeit neurologischer Erkrankungen beim Rind. SAT 139, 354-362, 1997

Fatzer R. und M.Vandevelde: Transmissible spongiforme Enzephalopathien (TSE) bei Tieren. Wiener Med.Wschr. 148, 78-85, 1998

Vandevelde M: Inflammatory diseases. In Pathobiology of the aging dog. ILSI Monographs. In Press 1998

Ehrensperger F., Vandevelde M: Neuropathologie der Transmissiblen spongiformen Enzephalopathien bei Tieren. In: BSE und andere spongiforme Enzephalopathien. Braun ed. Parey, Berlin 1998

Tipold A., Moore P, Jungi TW, Sager H, Vandevelde M : Lymphocyte subsets and CD45RA positive-Tcells in normal canine spinal fluid. J. Neuroimmunol. 82, 90-95, 1998

Theil D, Fatzer R, Schiller I, Caplazi P, Zurbriggen A, Vandevelde M: Neuropathological and aetiological studies of sporadic non-suppurative meningoencephalomyelitis of cattle. Veterinary record 143: 244-249, 1998

Burgener I, Van Ham L., Jaggy A., Vandevelde M, Tipold A: Chemotactic activity and IL-8 levels in the cerebrospinal fluid in canine steroid responsive meningitis-arthritis. J. Neuroimmunol. 89: 182-190, 1998

Braun U, Amrein E, Estermann U, Egli J, Schweizer T, Lutz H, Ehrensperger F, Vandevelde M, Kihm U: Untersuchungen an 182 Nachkommen von an Boviner Spongiformer Enzephalopathie erkrankten Kühen in der Schweiz. Teil I: klinische Befunde. Schweiz. Arch. Tierheilk 140: 240-249, 1998

Fatzer R, Ehrensperger F, Heim D, Schmidt J, Braun U, Vandevelde M: Untersuchungen an 182 Nachkommen von an Boviner Spongiformer Enzephalopathie erkrankten Kühen in der Schweiz. Teil 2: Epidemiologische und pathologische Befunde. Schweiz. Arch. Tierheilk 140: 250-254, 1998

Vandevelde M.: Pseudorabies. In: Clinical Microbiology and Infectious Diseases of the Dog and Cat. C.Greene ed. W.B. Saunders, Philadelphia, 1984 Uebersetzte Versionen 1990, 1998

Vandevelde M.: Central nervous disorders of suspected infectious etiology. In: Clinical Microbiology and Infectious Diseases of the Dog and Cat. C. Greene ed . W. B. Saunders , Philadelphia, 1984 Uebersetzte Versionen 1990, 1998

Tipold A, Moore P, Zurbriggen A, Burgener I, Barben G, Vandevelde M: Early T-cell response in the central nervous system in canine distemper virus infection. *Acta Neuropathol.* 97: 45 –56, 1999

Schobesberger M., Zurbriggen A., Summerfield A., Vandevelde M., Griot C.: Oligodendroglial degeneration in distemper: apoptosis or necrosis? *Acta Neuropathol.* 97: 179-187, 1999

Theil D, Fatzer R, Meyer R, Schobesberger M, Zurbriggen A and Vandevelde M: Nuclear DNA fragmentation and immune reactivity in bovine spongiform encephalopathy. *J. Comp. Pathol.* 121:357-367. 1999.

Meyer, R. K., B. Oesch, R. Fatzer, A. Zurbriggen, and M. Vandevelde: Detection of Bovine Spongiform Encephalopathy-Specific PrP(Sc) by Treatment with Heat and Guanidine Thiocyanate. *J. Virol.* 73:9386-9392, 1999.

Doherr M, Heim D, Vandevelde M, Fatzer R: Modelling the expected number of preclinical and clinical cases of bovine spongiform encephalopathy in Switzerland. *Vet.Rec.* 145, 1999, 155-160

Schaller O, Fatzer R, Stack M, Clark J, Cooley W, Biffiger K, Egli S, Doherr M, Vandevelde M, Heim D, Oesch B, Moser M: Validation of a western immunoblotting procedure for bovine PrP Sc detection and its use as a rapid surveillance method for the diagnosis of bovine spongiform encephalopathy. *Acta Neuropathol* 98: 437-443, 1999

Braun U, Amrein E, Estermann U, Pusterla N, Schönmann M, Schweizer, ehrensperger F, Vandevelde M, Kihm U: Reliability of a diagnosis of BSE made on the basis of clinical signs. *Veterinary record* 145: 198-200, 1999

Doherr MG, Oesch B, Moser M, Vandevelde M, Heim D: Targeted surveillance for bovine spongiform encephalopathy. *Veterinary record* 145, 672, 1999

Tipold A; Fatzer R; Jaggy A; Moore P; Vandevelde M. Presumed immune-mediated cerebellar granular degeneration in the Coton de Tulear breed. *J Neuroimmunol* 2000 110(1-2):130-3

Oesch B; Doherr M; Heim D; Fischer K; Egli S; Bolliger S; Biffiger K; Schaller O; Vandevelde M; Moser M. Application of Prionics Western blotting procedure to screen for BSE in cattle regularly slaughtered at Swiss abattoirs. *Arch Virol Suppl* 2000;(16):189-95

Meyer RK; Lustig A; Oesch B; Fatzer R; Zurbriggen A; Vandevelde M. A monomer-dimer equilibrium of a cellular prion protein (PrP^C) not observed with recombinant PrP. *J Biol Chem* 2000 275(48):38081-7

Schulz-Schaeffer WJ; Fatzer R; Vandeveld M; Kretzschmar HA. Detection of PrP(Sc) in subclinical BSE with the paraffin-embedded tissue (PET) blot. Arch Virol Suppl 2000;(16):173-80

Cherpillod P, Tipold A, Griot-Wenk M, Cardozo C, Schmid I, Fatzer R, Schobesberger M, Zurbriggen R, Brückner L, Roch F, Vandeveld M, Wittek R, Zurbriggen A: DNA vaccine encoding nucleocapsid and surface proteins of wild type canine distemper virus protects its natural host against distemper. Vaccine 18: 2927-2936,2000

Fatzer R, Gandini G, Jaggy A, Doherr M, Vandeveld M: Necrosis of Hippocampus and Piriform lobe in 38 domestic cats with seizures: a retrospective study on clinical and pathological findings. J Vet Intern Med, 14: 100-104, 2000

Tipold A, M.Vandeveld, R.Wittek, P.Moore, A. Summerfield and A.Zurbriggen: Partial protection and intrathecal invasion of CD8+ T cells in acute canine distemper virus infection. Vet. Microbiol. 83: 189-203, 2001

Tipold A; Vandeveld M; Wittek R; Moore P; Summerfield A; Zurbriggen A. Partial protection and intrathecal invasion of CD8(+) T cells in acute canine distemper virus infection. Vet Microbiol 2001 83(3):189-203

Demierre S; Tipold A; Griot-Wenk ME; Welle M; Vandeveld M; Jaggy A. Correlation between the clinical course of granulomatous meningoencephalomyelitis in dogs and the extent of mast cell infiltration. Vet Rec 2001 148(15):467-72

Vandeveld M, Fatzer R., Guarda F: Sistema nervoso. In Trattato di anatomia patologica veterinaria. Guarda&Mandelli ed. pp 695 – 734 UTET Torino. 2002

Vandeveld M, Jaggy A, Lang J.: Veterinärmedizinische Neurologie. Ein Leitfaden für Studium und Praxis. Parey Berlin 2001

Schobesberger M, Zurbriggen A, Doherr MG, Weissenböck H, Vandeveld M, Lassmann H, Griot C: Demyelination precedes oligodendrocyte loss in canine distemper virus-induced encephalitis. Acta neuropathol 103: 11-20, 2002

Gruber AD; Wessmann A; Vandeveld M; Summers BA; Tipold A. Mitochondriopathy with regional encephalic mineralization in a Jack Russell Terrier. Vet Pathol 2002 39(6):732-6

Callanan JJ; Mooney CT; Mulcahy G; Fatzer R; Vandeveld M; Ehrensperger F; McElroy M; Toolan D; Raleigh P. A novel nonsuppurative meningoencephalitis in young greyhounds in Ireland. Vet Pathol 2002 39(1):56-65

Fatzer R; Vandeveld M; Gottstein B. Cerebral taeniid oncospherical lesions in two BSE suspects. Vet Rec 2002 150(2):46-7

Lassmann H; Reindl M; Rauschka H; Berger J; Aboul-Enein F; Berger T; Zurbriggen A; Lutterotti A; Bruck W; Weber JR; Ullrich R; Schmidbauer M; Jellinger K; Vandeveld M. A new paraclinical CSF marker for hypoxia-like tissue damage in multiple sclerosis lesions. Brain 126:1347-57. 2003

Nadine Meertens, Michael H. Stoffel, Pascal Cherpillod, Riccardo Wittek, Marc Vandeveld and Andreas Zurbriggen: Mechanism of reduction of virus release and cell-cell fusion in persistent canine distemper virus infection. Acta neuropathol. 106: 303-310. 2003

Griot C; Vandeveld M; Schobesberger M; Zurbriggen A. Canine distemper, a re-emerging morbillivirus with complex neuropathogenic mechanisms Anim Health Res Rev 2003 4(1):1-10

Vandeveld, M. Neurodegenerations in domestic animals: a new way forward. Vet J. 2003 Nov; 166(3):218-9

Vandeveld M. Molecular mechanisms in CNS injury-The importance of therapeutic modification of cytokine expression in the brains of domestic animals. Vet. J. : 168: 205-206, 2004

Siso S, C. Navarro, D. Hanzlíček and M. Vandeveld. Adult onset thalamocerebellar degeneration in dogs associated to neuronal storage of ceroid lipopigment. Acta Neuropathol 108:386 - 392, 2004

Stein VM, Czub M, Schreiner N, Moore PF, Vandeveld M, Zurbriggen A, Tipold A : Microglial cell activation in demyelinating canine distemper lesions. J Neuroimmunol 153 (2004) : 122-131

Vandeveld M, Zurbriggen A: Demyelination in canine distemper virus infection: a review. Acta Neuropathol 109: 56-68, 2005

Sisó S, Botteron C, Muhle A, Vandeveld M.: A novel leukodystrophy in a dog. J Comp Path 2005, 132:232-236.

Tipold A, Vandeveld M.: Neurologic diseases of suspected infectious origin and Prion disease, meningoencephalitis. In: Infectious Diseases of the Dog and Cat. C. Greene ed . Elsevier St Louis, 2006, 795-806

Vandeveld M.: Pseudorabies. In Infectious Diseases of the Dog and Cat. C. Greene ed . Elsevier St Louis, 2006: 183-186

Vandeveld M. : Klassifikation von neurologischen Krankheiten: VETAMIN D. In Atlas und Lehrbuch der Kleintierneurologie. A. Jaggy ed. Schlütersche, 2005: 48 – 53

Siso S, Hanzlicek D, Fluehmann G, Kathmann I, Tomek A, Papa V, Vandeveld M: Neurodegenerative diseases in domestic animals: a comparative review. Vet.J 2006, 171: 20-38,

Tipold A, Vandeveld M: Central European tick-borne encephalitis. In infectious diseases of the dog and the cat, 3th edition. Greene ed. Elsevier, St.Louis, 195 (2006)

Stein, V.M., Baumgartner, W., Kreienbrock, L., Zurbriggen, A., Vandeveld, M., Tipold, A., 2006. Canine microglial cells: stereotypy in immunophenotype and specificity in function? Vet. Immunol. Immunopathol. 113, 277-287.

Fluehmann G, Konar M, Jaggy A, Nicollier A, Vandeveld M. Cerebral cholesterol granuloma in a cat. J Vet Intern Med 2006, 20: 1241-1244

Forterre F, Tomek A, Konar M, Jaggy A, Vandeveld M, Howard J. Multiple meningiomas: clinical, radiological, surgical, and pathological findings with outcome in four cats. J Feline Med Surg. 2006

Sisó S, Doherr MG, Botteron C, Fatzer R, Zurbriggen A, Vandeveld M, Seuberlich T. .Neuropathological and molecular comparison between clinical and asymptomatic bovine spongiform encephalopathy cases. Acta Neuropathol. 2007 114:501-508.

Wiener D, Plattet P, Cherpillod P, Zipperle L, Doherr MG, Vandeveld M, Zurbriggen A. Synergistic inhibition in cell-cell fusion mediated by the matrix and nucleocapsid protein of canine distemper virus. Virus Res. 2007 129:145-54.

Plattet P, Cherpillod P, Wiener D, Zipperle L, Vandeveld M, Wittek R, Zurbriggen A. Signal peptide and helical bundle domains of virulent canine distemper virus fusion protein restrict fusogenicity. J Virol. 2007 81:11413-25

Stein VM, Baumgärtner W, Schröder S, Zurbriggen A, Vandeveld M, Tipold A. Differential expression of CD45 on canine microglial cells. J Vet Med A Physiol Pathol Clin Med. 2007 54: 314-20.

Timmann D, Konar M, Howard J, Vandeveld M. Necrotising encephalitis in a French bulldog. J Small Anim Pract. 2007 48:339-42..

Wyss-Fluehmann G, Konar M, Jaggy A, Vandeveld M, Oevermann A. Cerebellar ependymal cyst in a dog. Vet Pathol. 2008;45:910-3

Stein VM, Schreiner NM, Moore PF, Vandeveld M, Zurbriggen A, Tipold A.
Immunophenotypical characterization of monocytes in canine distemper virus infection.
Vet Microbiol. 2008;131:237-46.

Tomek A, Forterre E, Konar M, Vandeveld M, Jaggy A. Intracranial meningiomas
associated with cervical syringohydromyelia in a cat. Schweiz Arch Tierheilkd.
2008;150(3):123-8.

Oevermann A, Bley T, Konar M, Lang J, Vandeveld M. A novel
leukoencephalomyelopathy of Leonberger dogs. J Vet Intern Med. 2008 ;22:467-71

Oevermann A, Botteron C, Seuberlich T, Nicolier A, Friess M, Doherr MG, Heim D,
Hilbe M, Zimmer K, Zurbriggen A, Vandeveld M. Neuropathological survey of fallen
stock: active surveillance reveals high prevalence of encephalitic listeriosis in small
ruminants. Vet Microbiol. 2008;130:320-9

Forterre F, Konar M, Tomek A, Doherr M, Howard J, Spreng D, Vandeveld M, Jaggy
A. Accuracy of the withdrawal reflex for localization of the site of cervical disk herniation
in dogs: 35 cases (2004-2007). J Am Vet Med Assoc. 2008;232:559-63

Timmann D, Cizinauskas S, Tomek A, Doherr M, Vandeveld M, Jaggy A.
Retrospective analysis of seizures associated with feline infectious peritonitis in cats. J
Feline Med Surg. 2008;10:9-15.

Antiepileptics

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Currently available anticonvulsants can be grouped into first-generation drugs, which were marketed before 1975, and new drugs, i.e., drugs which were marketed 1989 or later. Although since 1989 nine new drugs have been marketed, the progress made especially in treatment-resistant patients can be only considered incremental. Therefore, the need for new anticonvulsants which are effective in treatment-resistant patients and which are better tolerated still exists.

1. Introduction

Epilepsy is a condition of recurrent, paroxysmal seizures. The epileptic seizure represents an abnormal synchronized discharge among a large population of central neurons. Depending on the neuronal populations involved, a disturbance of movement, sensation, behavior, or consciousness can result from the discharges [1]. However, a seizure can only be seen as a symptom which principally can be induced in every individual if strong enough stimulation is applied. Epilepsy is characterized by the increased susceptibility to experience seizures upon exogenous or endogenous stimuli. Clinically, only a patient who has experienced three or more seizures is considered to be epileptic [2].

About 0.5 – 1 % of the world's population (i.e., more than 50×10^6 people) suffer from

epilepsy, which makes it one of the most common neurological disorders; no major difference is found between developed and developing countries [3]. The disease usually starts in childhood with a second peak in elderly people, but people can become epileptic at any age. Once acquired, patients usually suffer from the disease throughout their lives [3]. Epilepsy carries an increased risk of premature mortality. One study reported that patients who had not been seizure-free in the previous years had a 23-fold increased risk of sudden unexpected death relative to those patients with controlled seizures [4].

The diagnosis of epilepsy is based on clinical observations of the seizures and on recording of changes in the electrical activity of the brain using scalp electrodes (electroencephalography, EEG). Since patients in most cases lose consciousness during seizures and since EEG recordings between seizures can be fully normal, a preliminary diagnosis is often based on seizure descriptions obtained from eye witnesses. Ancillary investigations exploring the etiology of the condition include different imaging techniques, seizure provocation experiments, and depth electrode recordings. Epileptic seizures can be distinguished from other convulsive-like states not involving synchronous activation of neuronal cells. Examples of these states include transient ischemic attacks or hyperventilation resulting in loss of righting reflexes due to fainting.

The pathophysiology of epilepsy is diverse, involving both intrinsic and extrinsic factors. Developmental or congenital anomalies such as neuronal dysplasia, vascular malformations, and cerebral atrophy as well as cerebral tumors, meningitis, encephalitis or cerebrovascular diseases are possible intrinsic factors. Birth trauma, head injuries, anoxic disturbances induced by respiratory arrest, intoxication with heavy metals or other toxins and endocrine disorders such as uremia, hypoglycemia, or hypocalcemia are exogenous factors [5]. However, in most patients the epilepsy is of idiopathic origin. The precise mechanism involved in the increased susceptibility to synchronized discharges is still unknown. However, based on experiments with anticonvulsant and proconvulsant drugs and on biochemical findings from human epileptic tissue, several theories have been proposed. The increased excitability could be related to both neurotransmitter and ion channel function. An overactive excitatory and a reduced inhibitory neurotransmitter system has been found in these tissues. Reported changes in ion channel function resulted in a diminished membrane potential and a defective repolarization of the action potential. Besides these functional results, several genetic changes have been reported in both epilepsy-prone animals and in subpopulations of humans with epilepsy leading to hereditary epileptic syndromes [6, 7].

1.1. Classification

Seizures are stereotyped within individuals, but vary considerably between patients. This necessitates a system of classification for both seizures (symptomatic classification) and epileptic syndromes (syndromatic classification). The most generally accepted classification system is that of the International League Against Epilepsy [8, 9]

Classification of Seizures. Seizures are classified as to whether their onset is partial (focal) or generalized.

Partial seizures are further subdivided according to whether consciousness is retained throughout the seizure (simple partial seizures) or impaired at some point (complex partial seizures). Patients having *simple partial seizures*

show jerks or convulsions on one extremity. During *complex partial* seizures, the patient is in a state of reduced consciousness, not fully recognizing his surroundings, experiencing hallucinations and performing automatic gestures, such as talking, fumbling with clothing, or walking around. Any partial seizure can become *secondarily generalized* into a tonic – clonic convulsion.

Generalized seizures are further subdivided according to whether only consciousness is lost (absence seizures) or muscle activity is involved (myoclonic, clonic, tonic, tonic – clonic, or atonic seizures). An *absence seizure* is characterized by a sudden interruption of consciousness lasting only a few seconds without any other symptoms or with only a quick jerk of the eyes or hands. *Myoclonic seizures* are characterized by sudden jerks on the musculature of the limbs, the head, or the whole body, appearing in connection with absence seizures or as a separate entity. During an *atonic seizure* patients exhibit a sudden loss of muscle tone resulting in a fall to the ground (drop attack). Consciousness may be lost but is immediately regained. *Tonic-clonic seizures* start with a sudden loss of consciousness and a fall to the ground; a few seconds of intense tonic spasms of major muscles are followed by generalized jerks of the whole body. These convulsions gradually cease, leaving the patient comatose and flaccid. A gradual recovery of consciousness follows, often combined with confusion and drowsiness. *Status epilepticus* is a condition in which seizures follow each other without a recovery of consciousness between them. Both absence seizures and convulsive seizures can occur as status epilepticus.

Classification of Syndromes. A classification of epileptic syndromes is based not only on seizure characteristics but on more general features of the disease such as age of onset, type of EEG abnormalities, associated neurological features, or possible cause of disease including genetic factors [10, 11]. The International League Against Epilepsy [8, 9] has listed more than 30 different epileptic syndromes.

1.2. Treatment of Epilepsy

The treatment of epilepsy is based on pharmacotherapy to suppress the seizure attacks, be-

havioral therapy to avoid seizure initiating situations, and on surgical resection of the seizure focus. As yet no ideal and general treatment regime exists for epileptic patients; the therapy rather has to be adopted individually. With optimal usage of the aforementioned strategies, seizures can be fully controlled in about 70–80 % of patients, however, side effects often have to be accepted [12]. Although surgical resection of the hyperexcitable focus is considered the only available cure for epilepsy, only a small percentage of patients, i.e., those having a defined singular focus which can be resected without disturbing vital brain functions, is eligible for surgery. Based on this well-defined population, the success rate of surgery is high but serious side effects can occur such as unwanted changes in personality or loss of brain functions like short term memory or ability to speak [13]. Behavioral treatment is aimed at avoiding endogenous or exogenous stimuli and is therefore only of symptomatic nature. Generally, the same is also true for pharmacotherapy, which is aimed at suppressing the seizure attacks. The pharmacotherapy must be adapted both to seizure type and epileptic syndrome.

Antiepileptics (anticonvulsants) can be divided into drugs active against generalized absence seizures, those against partial seizures, and in drugs with a broad spectrum of activity. Since seizures are often resistant to pharmacotherapy, two or more anticonvulsant drugs are concomitantly administered to improve seizure control [2, 14]. As of 2007, a syndrome-related therapy is becoming increasingly relevant with anticonvulsants assigned to treatment of specific syndromes. However, drugs with marketing authorization for specific syndromes are not necessarily specific for the treatment of such syndromes. Instead, the risk–benefit ratio, especially in severe childhood syndromes such as Lennox-Gastaut syndrome, enables the use of anticonvulsants for the treatment of these syndromes while they could not be used in a broad spectrum of patients due to risk of severe side effects. Examples of such drugs with broad spectrum potent activity but syndrome-specific clinical use are vigabatrin, which induces visual field defects in up to 50% of patients [15], or elbamate, which is afflicted with the risk of aplastic anaemia and hepatic failure [16]. Another reason for the development of an anticonvulsant

for the treatment of epilepsy syndromes is the possibility to utilize fast development strategies since syndrome-specific treatments often fulfil the criteria for orphan drug status. This status enables rapid development of drugs for rare severe diseases and grants additional market exclusivity. One recent example is stiripentol which was recently marketed under orphan drug status in the EU for the treatment of severe myoclonic epilepsy in infancy [17].

1.3. Discovery Strategies for New Antiepileptic Drugs

Discovery strategies of new antiepileptic drugs focus on compounds which are effective especially in patients which can not be satisfactorily treated with currently available medication, i.e., which are drug-resistant. As a second goal, new AEDs should be better tolerated and should be easy to use. Since the pathomechanisms of epilepsy and especially of drug resistance are not fully understood, it is impossible to define a single straightforward strategy to develop drugs aimed at a molecular target which is solely and critically involved in generation of seizures. Therefore, nonrational (older) strategies of drug development are still valid in this field [18, 19].

Serendipitous search for new drugs, based on anticonvulsant effects observed in in vivo or in vitro screening models of seizures, may lead to drugs with unknown modes of action but potent anticonvulsant activity. This approach, however, is limited by the animal models used [20]. If models are selected based on efficacy of known drugs, only drugs with a similar anticonvulsant profile might evolve. If models are selected to mimic the disease state or drug resistance [21], the predictive value might still be limited. While a number of models with proposed predictivity for drug resistance are described including the phenytoin- or lamotrigine-resistant kindled rat and the 6-Hz-psychomotor seizure model of partial epilepsy, the predictive value of these models still awaits clinical validation [22]. A different successful strategy to find new marketable drugs which are better than existing ones is to optimize the structure of known anticonvulsants. This can be done using predictive animal models and structure–activity relationship (SAR) modelling to improve anticonvul-

sant potency while reducing unwanted effects. Often the optimization of metabolic properties of known drugs to prevent the generation of toxic metabolites is target for chemical modification. Examples of such structural optimizations are fluorofelbamate [23], eslicarbazepine [24], and different valproate derivatives [25]. While this strategy may result in compounds with reduced side effects, it is unlikely to obtain drugs which a new activity profile, targeting drug resistant patients.

A more rational approach to new antiepileptic drugs and especially an approach focusing on individual molecular targets is hampered by two important factors: Heterogeneity of epileptic syndromes and lack of exact understanding of the cause of epilepsy. Molecular targets can principally be derived from at least three sources [26]. The first approach is to consider the molecular targets of synthetic or synthetic proconvulsant agents. This yields mostly targets which are already addressed by current anticonvulsants, i.e., the GABA_A receptor, Na⁺ and Ca⁺ channels, as well as glutamate receptors. Other convulsants block potassium channels. Indeed, first K⁺ channel-activating drugs including retigabine [27] are under development but other K⁺ channels may also serve as interesting targets. Finally, many metabolic poisons can induce seizures and it may be interesting to study the pathophysiology of such seizures. A related approach is to identify the mechanism of action of compounds with anticonvulsant activity. Indeed, this approach has yielded a number of interesting targets. The first drug taking this approach was retigabine [28] identifying the Kv7 channel as new target. More recently the discovery of the mechanism of action of gabapentin, i.e., the interaction with the $\alpha 2\delta$ calcium channel subunit, has lead to the development and marketing of pregabalin [29]; furthermore, the identification of the synaptic vesicle protein (SV2A) as molecular target of levetiracetam has resulted in the rational design of two new drug candidates which are in clinical testing, i.e., breviracetam and seletracetam [30, 31]. While seletracetam was found to be selective for the SV2A protein, breviracetam combines this activity with a use dependent block of neuronal sodium channels, i.e., with a target of many well-established drugs. This combination makes breviracetam an interesting candidate, with the potential for broad-

spectrum anticonvulsant activity. However, the interaction with sodium channels may also result in typical side effects of sodium channel blockers and the clinical benefit of this dual mechanism is to be shown.

The second approach to identifying novel AED targets is to select from among the cellular elements that have a physiological role in generation of rhythmic discharges and epileptic phenomena in model preparations including human tissue. While this approach identifies all the ion channels and receptors mentioned above, it in addition suggests some unexpected targets. These include gap-junction proteins (connexins), hyperpolarization-activated cation channels and neurotrophins. [26]. While preclinical evidence supports the role of these target in epilepsy and while first compounds are available, no drug has reached clinical development.

The third rational approach to defining new AED targets is to consider as candidates the protein products of genes associated with epilepsy syndromes in humans as well as in animals [26]. The genes of most hereditary epilepsy syndromes in man have been identified and in many cases the way in which gene mutation alters function has been established. The genes identified so far encode predominantly ion channels, which are in many cases identical to targets of empirically identified drugs or which were proposed as targets utilizing the above mentioned rational target discovery strategies.

2. Antiepileptic Drugs

Landmarks in Antiepileptic Drug Treatment:

1857	Bromides
1912	Phenobarbital
1938	Phenytoin
1958	Ethosuximide
1967	Carbamazepine
1974	Valproic acid
1989	Vigabatrin
1989	Zonisamide
1991	Lamotrigine
1993	Gabapentin
1995	Topiramate
1996	Tiagabine
2000	Levetiracetam
2004	Pregabalin
2007	Stiripentol

Phenobarbital was the first effective organic antiepileptic agent and the starting point for structural variation leading to hydantoins (phenytoin), succinimides (ethosuximide), and oxazolidinediones (trimethadione). These active chemical classes exhibit certain structural similarities, they all have an imide group and a carbon atom bearing four nonhydrogen substituents. In spite of chemical similarities, the structural variation leads to different anticonvulsant effects. Like phenobarbital, phenytoin is highly effective in suppressing generalized tonic-clonic as well as simple complex partial seizures, and ethosuximide is effective in suppressing absence seizures. Although structural similarities exist between these classes, phenytoin and ethosuximide exert the activity through different mechanisms. Thus, phenytoin blocks voltage dependent Na^+ -channels and ethosuximide blocks Ca^{2+} -T-channels.

Therefore, structure-activity relationships can be discussed only within well-defined chemical classes: hydantoins [32], dibenz[b,e]azepines [33], acetic acids [34], succinimides [35], oxazolidinediones [36], barbiturates [37], benzodiazepines [38] or for compounds of different chemical classes which exert anticonvulsant activity essentially through the same mechanism [39, 40].

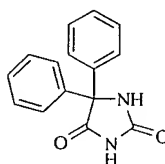
Current therapeutic use is the criterion for listing a compound in this article. Since the 1980s several new antiepileptic drugs have entered the market. At present the new drugs are an alternative to the old drugs for an increasing number of patients with refractory epileptic seizures. The search for effective and cheap drugs which are well tolerated and devoid of side effects still persists. Books on the old and new antiepileptic drugs [41, 42] and reviews of the new antiepileptic drugs [43, 44] have appeared, additional mechanistic aspects are discussed in [45]. The market value of antiepileptics has grown significantly. Worth \$ 0.56×10^9 in 1987, the market topped \$ 8×10^9 in 2003 and, if current growth rates continue, could exceed \$ 14×10^9 by 2012 (currently, 50 % of the antiepileptic drugs are sold in North America). Much of this spectacular growth is due to the premium-priced products entering the market. The well-established and cheaper drugs (phenytoin, carbamazepine, and valproate) covered in 2003 about 30 % of the market. The share of the

anticonvulsant market attributed to epilepsy prescriptions has decreased because of growing use in other areas, predominantly neuropathic pain, bipolar disorder, and migraine. [46].

2.1. Phenytoin

Phenytoin [57-41-0], 5,5-diphenyl-2,4-imidazolidinedione, $\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}_2$, M_r 252.26, mp 295 – 298 °C; practically insoluble in water. 1 g dissolves in about 75 mL of ethanol or 30 mL of acetone.

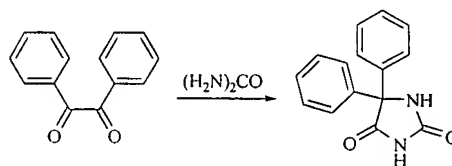
Phenytoin sodium [630-93-3], sodium 5,5-diphenylhydantoin, $\text{C}_{15}\text{H}_{11}\text{NaN}_2\text{O}_2$, M_r 274.26; 1 g dissolves in ca. 66 mL of water. The aqueous solution is turbid unless the pH is adjusted above 11.7, which is the pH of the saturated solution. 1 g dissolves in 10.5 mL of alcohol. It is practically insoluble in ether and chloroform.



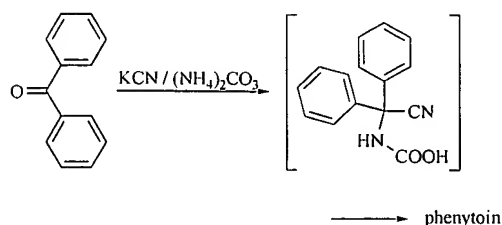
Trade names: Dilantin (Mylan Laboratories), Epanutin (Pfizer)

Synthesis:

- 1) Condensation of benzil with urea [47]



- 2) Bucherer reaction [48]



Phenytoin and its sodium salt are effective in primarily and secondarily generalized tonic-clonic seizures and are also indicated in simple and complex partial seizures, but not in absence seizures. The intravenous form can be used in status epilepticus. The clinical use of phenytoin is complicated by its narrow therapeutic margin and its saturation kinetics. Side effects include nystagmus, ataxia, slowly progressing impairment of mental function, skin rash, gingival hyperplasia, and hirsutism. The main mechanism of action of phenytoin is based on the blockade of voltage-dependent Na^+ -channels.

The share of Dilantin in the world market was 2 % in 2003.

Other Hydantoins.

Mephenytoin [50-12-4], 5-ethyl-3-methyl-5-phenyl-2,4-imidazolidinedione, can be substituted for phenytoin where the peripheral neuropathy and hirsutism create problems. Side effects, including rash, fever, and fatal blood dyscrasia, prevent the use as an anticonvulsant drug of first choice.

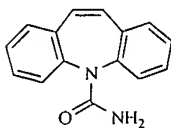
Trade name: Insulton (Sandoz).

Ethotoin [86-35-1], 3-ethyl-5-phenyl-2,4-imidazolidindione, has never achieved the status of a drug of choice.

Trade name: Peganone (Abbott).

2.2. Carbamazepine and Oxcarbazepine

Carbamazepine [298-46-4], 5*H*-dibenz[*b,f*]azepine-5-carboxamide, $\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}$, M_r 236.26, *mp* 189–193 °C; soluble in chloroform, DMF, ethylene glycol monomethyl ether, or methanol, only slightly soluble in ethanol or glacial acetic acid, virtually insoluble in water.



Trade names: Tegretol, Carbatrol (Novartis); others, e.g., Fokalepsin (Lundbeck), Sirtal (Sanofi), Timonil (Desitin).

Synthesis: 5*H*-dibenz[*b,f*]azepine is first reacted with phosgene and then with ammonia [49].

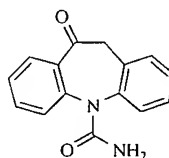
Carbamazepine is the only antiepileptic combining structural characteristics of classical antiepileptics with those of polycyclic psychoactive agents, such as imipramine (→ Psychopharmacological Agents).

Carbamazepine is the drug of choice for complex partial seizures and is also effective against simple partial and generalized tonic-clonic seizures, but not against absence seizures. Carbamazepine also has an antineuralgic and a psychotropic effect, i.e., a positive influence on disturbed mood and behavior, and little or no detrimental effect on intellectual functions. Side effects include skin rash, headache, nausea, fatigue, vertigo, and ataxia.

The substance is considered to exert its main effect by inhibiting voltage-dependent Na^+ -channels.

The share of Tegretol and Carbatrol in the world market was 4.3 % in 2003.

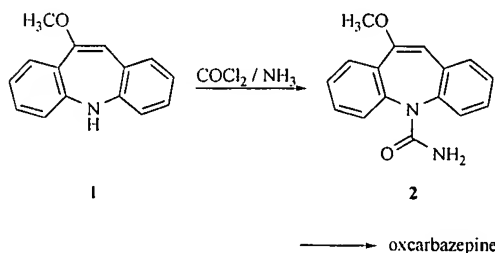
Oxcarbazepine [28721-07-5], 10,11-dihydro-10-oxo-5*H*-dibenz[*b,f*]azepin-5-carboxamide, $\text{C}_{15}\text{H}_{12}\text{N}_2\text{O}_2$, M_r 252.27, *mp* 215–216 °C; virtually insoluble in water.



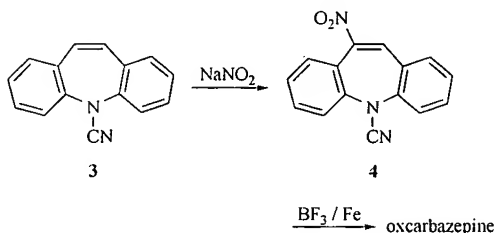
Trade names: Trileptal (Novartis), Timox (Desitin).

Synthesis: Oxcarbazepine can be obtained in different ways.

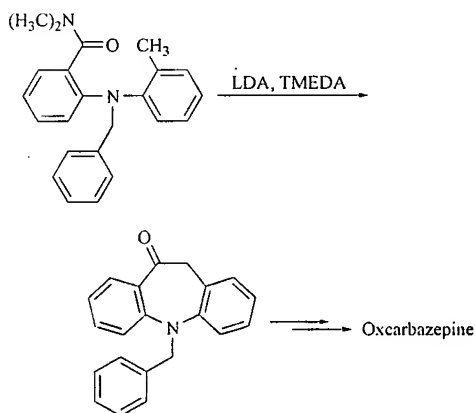
- 1) Reaction of 10-methoxy-5*H*-dibenz[*b,f*]azepine (1) with phosgene gives the 5-chlorocarbonyl compound, treatment with NH_3 affords 10-methoxy-5*H*-dibenz[*b,f*]azepine-5-carboxamide (2), which is hydrolyzed with diluted HCl to oxcarbazepine [50].



- 2) Nitration of 5-cyano-5*H*-dibenz[*b,f*]azepine (3) with NaNO₃ in acetic anhydride/acetic acid gives 5-cyano-10-nitro-5*H*-dibenz[*b,f*]azepine (4), which is treated with BF₃ and powdered iron in acetic acid [51].



- 3) The synthesis of intermediate **A** and the following cyclization by means of LDA and TMEDA in THF affords **B**. The deprotection of **B** gives 10,11-dihydro-5H-dibenz[b,f]azepin-10-one, which is finally treated with chlorosulfonyl isocyanate to afford oxcarbazepine [46].



Oxcarbazepine is a new antiepileptic drug (launched in 1990). Compared with its parent drug, carbamazepine, it is metabolized via a different pathway and may have milder side effects. The efficacy of the two drugs seems to be similar.

The share of Trileptal in the world market was 5.1 % in 2003.

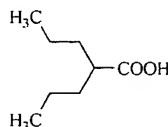
2.3. Valproic Acid

Valproic acid [99-66-1], 2-propylpentanoic acid, $C_8H_{16}O_2$, M_r 144.21; colorless liquid, *bp* 221

– 222 °C at 101.3 kPa, $n_D^{25} = 1.425$, $d_4^{25} = 0.904$; soluble in most organic solvents, including methanol, chloroform, and ether, solubility in water: 1.27 mg/mL.

Sodium valproate [1069-66-1], $\text{C}_8\text{H}_{15}\text{NaO}_2$, M_r 166.20, colorless crystalline powder, hygroscopic, very soluble in water (ca. 0.66 g/mL of solution), soluble in ethanol (ca. 0.2 g/mL of solution), practically insoluble in chloroform and diethyl ether.

Calcium valproate [138995-18-3],
C₁₆H₃₀CaO₄ · 2H₂O, *M*_r 362.5



Trade names: Depakene, Depakote, Valcote (Abbott); others, e.g. Convulex (Lundbeck), Mylproin, Orfiril (Desitin), Convulsofin (Calsalt, AWD.pharma).

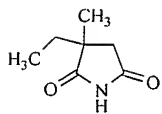
Synthesis: Diethyl malonate is alkylated with 1-bromopropane and then hydrolyzed and decarboxylated [53].

Valproic acid and its salts are major antiepileptic drugs for the treatment of absence, myoclonic, and generalized tonic-clonic seizures and also may be effective against complex partial seizures. The most common side effects are gastrointestinal disturbances and sedation. The most serious adverse effect is liver damage, although this is rare. Teratogenic effects, predominantly spina bifida, are reported frequently. The mechanism of action of valproic acid is not certain. The most cited mechanisms are the increase in GABA function and the blockade of voltage-dependent Na^+ -channels.

The share of Depakote and Depakene in the world market was 17.6 % in 2003.

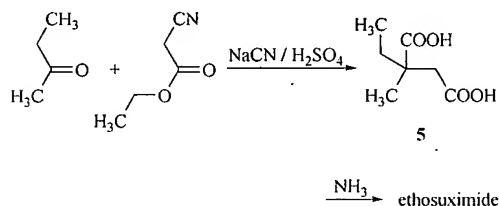
2.4. Ethosuximide and Trimethadione

Ethosuximide [77-67-8], 3-ethyl-3-methyl-2,5-pyrrolidinedione, $C_7H_{11}NO_2$, M_r 141.17, mp 64–65 °C; very soluble in water.



Trade names: Zarontin (Pfizer), Petnidan (Desitin), Suxilep (Parke-Davis)

Synthesis: The condensation of butanone with cyano ethyl acetate and subsequent reaction with NaCN and H₂SO₄ gives 2-ethyl-2-methylsuccinic acid (5), which is ring-closed via the diammonium salt to yield ethosuximide [54].



Ethosuximide is the most effective succinimide against absence seizures. It acts by blockade of T-type Ca²⁺-channels in thalamic neurons.

Ethosuximide is a useful and unique antiepileptic drug, but for the most forms of absences valproic acid is the drug of first choice.

Phensuximide [86-34-0], 1-methyl-3-phenyl-2,5-pyrrolidinedione

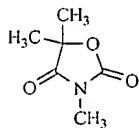
Trade name: Mironin (Parke – Davis).

Methsuximide [77-41-8], 1,3-dimethyl-3-phenyl-2,5-pyrrolidinedione

Trade name: Cerontin (Parke – Davis).

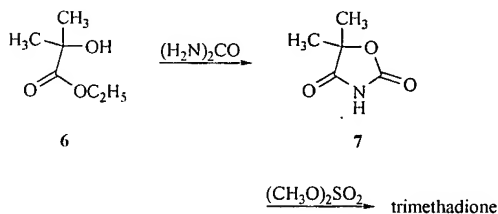
Phensuximide and methsuximide have also some efficacy against complex partial seizures.

Trimethadione [127-48-0], 3,5,5-trimethyl-1,3-oxazolidine-2,4-dione, C₆H₉NO₃, M_r 143.14, mp 46 – 46.5 °C; slight camphorlike odor, burning faintly bitter taste; solubility in water about 5 %, freely soluble in chloroform, ethanol, and diethyl ether.



Trade names: Tridione, Trimedone (Abbott).

Synthesis: Ethyl 2-hydroxy-2-methylpropionate (6), obtained by reaction of acetone and KCN, is condensed with urea in the presence of sodium ethoxide to give 5,5-dimethyl-1,3-oxazolidine-2,4-dione (7), which is methylated with dimethyl sulfate to form trimethadione [55].

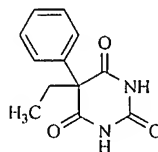


Trimethadione is effective against absence seizures, but its clinical use is very limited because of its toxicity.

The share of succinimide and oxazolidine drugs in the world market is <1 %.

2.5. Phenobarbital and Primidone

Phenobarbital [50-06-6], phenobarbitone, 5-ethyl-5-phenyl-2,4,6(1H,3H,5H)-pyrimidinetrione, C₁₂H₁₂N₂O₃, M_r 232.23; (synthesis and properties of phenobarbital and other barbiturates → Hypnotics, Section 5.1).



Trade names: Eskabarb (GSK), Lepinal, Luminal (Desitin), Phenobal (Fujinaga).

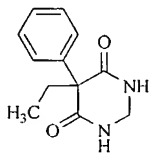
Phenobarbital is highly effective in suppressing generalized tonic – clonic as well as simple and complex partial seizures; however, its clinical use is limited because of its serious side effects: fatigue, drowsiness, irritability, and especially deterioration of higher cerebral functions (attention memory, and intellectual performance). Intravenous use in status epilepticus may lead to severe respiratory depression. Phenobarbital potentiates inhibitory GABAergic and inhibits excitatory glutamatergic neuro-

transmission through interaction with modulatory binding sites on the respective neurotransmitter receptors.

Methylphenobarbital [115-38-8], 5-ethyl-1-methyl-5-phenyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione, is regarded as a prodrug for phenobarbital and has an advantage over phenobarbital because it produces plasma phenobarbital levels that vary in direct proportion to drug dose.

Trade name: Mebaral (Sanofi-Aventis).

Primidone [125-33-7], 5-ethyl-5-phenyl-4,6(1*H*,5*H*)-pyrimidinedione, $C_{12}H_{14}N_2O_2$, M_r 218.25, *mp* 281–282 °C; slightly bitter taste; sparingly soluble in water (0.6 g/L at 37 °C) and most organic solvents.



Trade names: Liskantin (Desitin), Mylepsinum (AstraZeneca), Resimatil (Sanofi-Aventis).

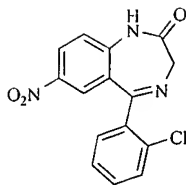
Synthesis: Primidone is obtained by reductive desulfurization of 2-thiophenobarbital with Raney nickel or zinc and formic acid, or by ring closure of ethylphenylmalonamide with formamide or formic acid [56].

Primidone is effective against most generalized and partial seizure types, but its clinical use is limited because of its serious side effects, which are similar to those of its main metabolite, phenobarbital. Although primidone is a prodrug for phenobarbital its antiepileptic properties are independent of phenobarbital.

Phenobarbital and primidone are rarely used worldwide (share < 1 % of the sales of antiepileptics).

2.6. Benzodiazepines (→ Hypnotics, Section 5.4, → Sedatives, Section 3.1, → Psychopharmacological Agents, Section 4.1)

Clonazepam [1622-61-3], 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepin-2-one, $C_{15}H_{10}ClN_3O_3$, M_r 315.72; *synthesis:* [57].



Trade name: Rivotril (Novartis).

The other 1,4-benzodiazepines, diazepam [439-14-5], 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one, *trade name:* Valium (Roche), (→ Psychopharmacological Agents) and nitrazepam [146-22-5], 1,3-dihydro-7-nitro-5-phenyl-2*H*-1,4-benzodiazepin-2-one, *trade name:* Mogadon (Roche), (→ Hypnotics, Section 5.4) as well as the 1,5-benzodiazepine clobazam [22316-47-8], 7-chloro-1-methyl-5-phenyl-1*H*-1,5-benzodiazepine-2,4(3*H*,5*H*)-dione, *trade name:* Frisium, (Hoechst), (→ Psychopharmacological Agents, Section 4.1.2) have been in clinical use for a long time.

The major clinical use of the benzodiazepines in epilepsy is in the initial treatment of status epilepticus. They are also effective in a variety of other seizure types, but tolerance to their effects usually develops early in the course of treatment. For this reason they are used mostly for adjunct therapy in refractory cases.

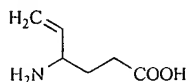
The antiepileptic activity of benzodiazepines is mediated primarily by an enhancement of GABAergic synaptic transmission.

The benzodiazepines are widely used (sales of Rivotril 0.8 % in 2003).

2.7. New Drugs

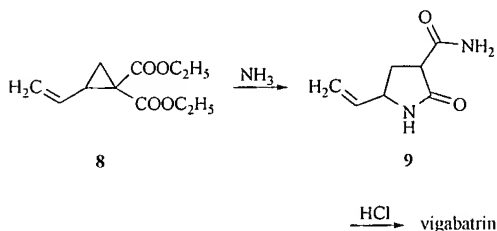
Since the 1980s several new drugs have been approved or are in the process of being approved, e.g., vigabatrin, felbamate, zonisamid, lamotrigine, gabapentin, topiramate, and tiagabine (oxcarbazepine see Section 2.2). At present, the main use of the new agents is in patients that are refractory to first-line drugs such as carbamazepine or valproate. Further studies are required to characterize their activity spectrum as well as their potential value in monotherapy [43, 44].

Vigabatrin [60643-86-9], 4-amino-5-hexenoic acid, $C_6H_{11}NO_2$, M_r 129.16, *mp* 171 – 177 °C; off-white crystals; solubility: water 335 mg/mL, ethanol 7.5 mg/mL, chloroform 0.1 mg/mL.



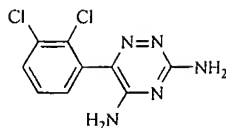
Trade name: Sabril (Merrell Dow), Sabrilex (AstraZeneca).

Synthesis: The reaction of 1,4-dichloro-2-butene with diethyl malonate in the presence of sodium ethoxide as catalyst in refluxing ethanol gives 1,1-bis(ethoxycarbonyl)-2-vinylcyclopropane (**8**), which is converted into 3-carboxamido-5-vinyl-2-pyrrolidone (**9**) by reaction with gaseous ammonia in DMF. This compound is treated with HCl in refluxing acetic acid to yield vigabatrin [58].



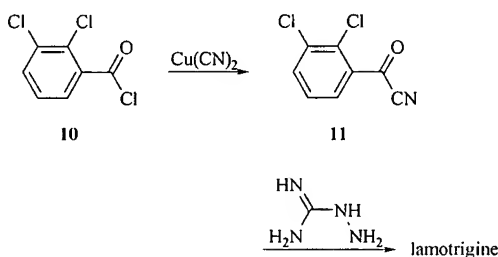
Vigabatrin has a good efficacy profile in partial seizures. However, the treatment is frequently associated with retinal toxicity resulting in visual field defects. In addition, the anticonvulsant efficacy is lost in up to 50 % of patients due to development of tolerance. Other side effects are weight gain and changes in mood and behavior. It has been found that vigabatrin potentiates the GABAergic transmission through inhibition of GABA-transaminase. Due to the irreversible retinal toxicity, vigabatrin is currently only used in few patients with otherwise intractable epilepsy syndromes and the use is based on a rigid case by case risk-benefit evaluation [15].

Lamotrigine [84057-84-1], 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine, $C_9H_7Cl_2N_2$, M_r 256.09, *mp* 216 – 218 °C; off-white crystals; pK_a 5.5.



Trade name: Lamictal, Bipolar, Labileno (GSK)

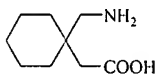
Synthesis: The reaction of 2,3-dichlorobenzoyl chloride (**10**) with $Cu(CN)_2$ and KI in refluxing xylene yields 2,3-dichlorobenzoyl cyanide (**11**), which is cyclized with aminoguanidine in DMSO [59].



Lamotrigine is considered to be effective in different seizure types and it is well tolerated. The main mechanism of action is the blockade of voltage dependent Na^+ -channels. Severe skin rashes can necessitate the stop of the treatment.

The share of Lamictal in the world market was 10.7 % in 2003.

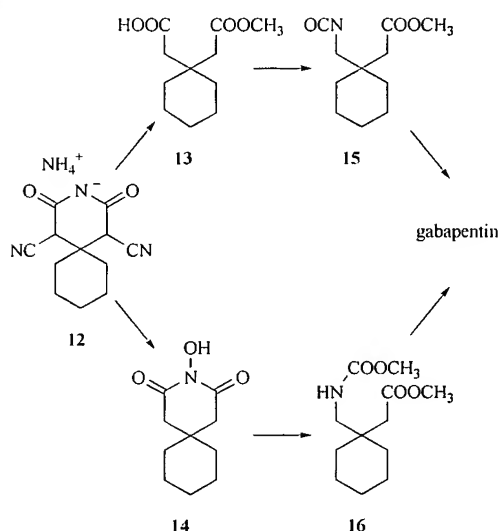
Gabapentin [60142-96-3], 1-(aminomethyl)cyclohexanecarboxylic acid, $C_9H_{17}NO_2$, M_r 171.24, *mp* 165 – 167 °C; off-white crystals; zwitterion at physiologic pH; highly water soluble.



Trade name: Neurontin (Pfizer), Gabax (Temmler)

Synthesis: The synthesis is started with the Guareschi salt (**12**) (obtained from cyclohexanone and cyanoacetate), which is hydrolyzed and decarboxylated to give 1,1-cyclohexanediacyetic acid. The anhydride can be treated either with methanol to yield the half ester (**13**) or with hydroxylamine to afford the *N*-hydroximide (**14**). The half ester is converted to the azide and subjected to a Curtius-

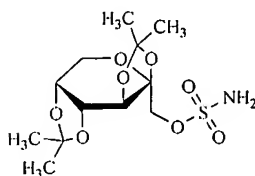
type rearrangement to give the isocyanate (15) which is hydrolyzed to gabapentin. Gabapentin is also obtained from the corresponding *N*-hydroxyimide (14) via a Lossen-type rearrangement by conversion of (14) to *N*-benzenesulfonyloxime and following reaction with triethylamine to the urethane ester (16) [60].



Gabapentin has a good tolerability. Dizziness, fatigue, somnolence, ataxia and tremor are relatively common. Its short half-life in the body is a disadvantage. Gabapentin binds with high affinity to a branched amino acid transporter. This may be the reason for increased GABA-levels in certain region of the brain during therapy with gabapentin.

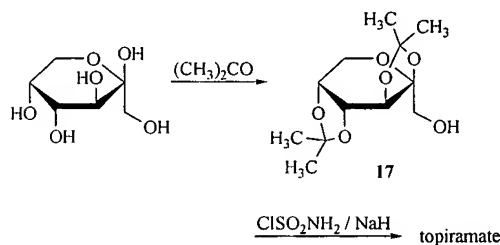
The share of Neurontin in the world market was 33.4 % in 2003, but the sales are decreasing.

Topiramate [97240-79-4], 2,3:4,5-bis-*O*-(1-methylethylidene)- β -D-fructopyranose sulfamate, $C_{12}H_{21}NO_8S$, M_r 339.36, mp 125 – 126 °C; $[\alpha]_D^{24} = -34.0$; soluble in methanol.



Trade names: Topamax (Johnson & Johnson), Epitamax, Topimax (Janssen-Cilag)

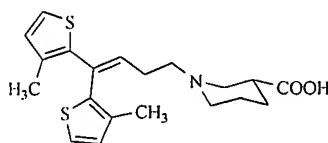
Synthesis: D-Fructose is reacted with acetone to produce the bisacetone (17). This compound is then condensed with sulfamoyl chloride in presence of sodium hydride [61].



Topiramate has a good efficacy profile in partial seizures and probably a broad spectrum. Its mechanism of action includes blockade of voltage-dependent Na^+ -channels, potentiation of GABA response, antagonism of Kainate/AMPA receptor sites, and inhibition of carbonic anhydrase. Clinically significant adverse effects associated with topiramate are fatigue, dizziness, somnolence, impaired concentration, ataxia, and weight loss.

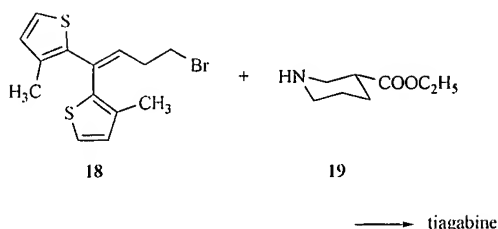
The share of Topamax in the world market was 13.1 % in 2003.

Tiagabine [115103-54-3], (*R*)-(-)-1-[4,4-bis(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid hydrochloride, $C_{20}H_{25}NO_2SO_2 \cdot HCl$, M_r 412.0, mp 193 – 195 °C; off-white, nonhygroscopic, crystalline powder; sparingly soluble in water.



Trade name: Gabitril (Abbott), Tiabex (Cephalon).

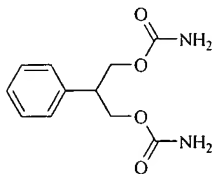
Synthesis: Bromobis(methylthienyl)butene (18) is reacted with *R*-(-)-ethyl-3-piperidine carboxylate (19) and subsequently hydrolyzed to give the free base which is converted to the hydrochloride [62].



Tiagabine is considered to show a similar activity as vigabatrin or lamotrigine against refractory partial seizures in randomized-controlled add-on trials. Tiagabine exerts its effect by enhancement of GABAergic transmission through inhibition of GABA reuptake. Dizziness, headache, tremor, impaired concentration, and fatigue are common side effects.

The share of Gabitril in the world market was 0.8 % in 2003.

Felbamate [25451-15-4], 2-phenyl-1,3-propanediol dicarbamate, $C_{11}H_{14}N_2O_4$, M_r 238.24, mp 150.3 – 151.2 °C; white crystalline powder; solubility in water 0.33 mg/mL, in ethanol 5.0 mg/mL, and in DMF 333.4 mg/mL.



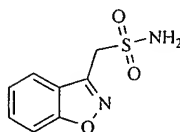
Trade names: Felbatol (Carter Wallace), Taloxa (Schering-Plough).

Synthesis: The synthesis of felbamate was first published in 1959. 2-Phenyl-1,3-propanediol is reacted with ethylcarbamate to yield felbamate [63].

Felbamate is effective in Lennox – Gastaut syndrome and it is considered to act by blockade of voltage-dependent Na^+ -channels, antagonism of excitatory transmission by selectively blocking NMDA receptors containing the NR2B subunit, and potentiation of GABA response. Felbamate, however, has been associated with a high risk of aplastic anemia and hepatotoxicity.

The share of Felbatol in the world market was 0.3 % in 2003.

Zonisamide [68291-97-4], 1,2-benzisoxazole-3-methane-sulfonamide, $C_8H_8N_2O_3S$, M_r 212.23, mp 164 – 168 °C; white to pale yellow crystals or crystalline powder; freely soluble in acetone, sparingly soluble in ethanol (95 %), soluble in water 0.8 mg/kg at pH 7.



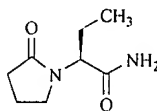
Trade name: Zonegran (Eisai).

Synthesis: The reaction of 3-bromomethyl-1,2-benzisoxazole with sodium sulfite in methanol/water gives sodium 1,2-benzisoxazole-3-methanesulfonate, which is converted into 1,2-benzisoxazole-3-methanesulfonyl chloride. Treatment with NH_3 gives zonisamide [64].

Clinical trials have shown zonisamide to be a promising drug for treating a wide variety of seizures. Studies on zonisamide's cellular mechanism of action have demonstrated that the drug blocks voltage-sensitive Na^+ - and Ca^{2+} -channels. Relatively common side effects are anorexia, ataxia, dizziness, fatigue, somnolence, impaired concentration, and confusion.

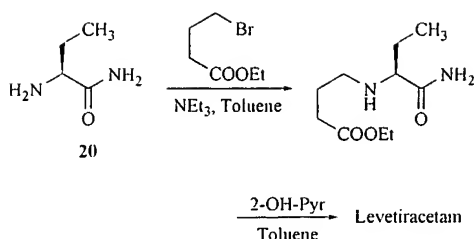
The share of Zonegran in the world market was 1.6 % in 2003.

Levetiracetam [102767-28-2], (-)-2(S)-(2-oxopyrrolidine-1-yl)butyramide, $C_8H_{14}N_2O_2$, M_r 170.21, mp 117 °C; white powder, readily soluble in water.



Trade name: Keppra (UCB).

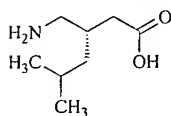
Synthesis: The syntheses start from (S)-2-aminobutyramide (20) or (S)-4-(methylthio)-2-aminobutyramide [52], or alternatively from 2-pyrrolidone, followed by a resolution of the appropriate racemate with (R)-(+)-2-methylbenzylamine [65] or the appropriate intermediate is submitted to an asymmetric hydrogenation over a chiral Rh catalyst [66].



Levetiracetam is an N-type calcium channel (Ca(v) 2.2) blocker and is used worldwide against different seizures, among others it is approved as a monotherapy for the treatment of partial onset seizures with or without secondary generalization in adults with newly diagnosed epilepsy. It is more effective in terms of responder rates than gabapentin and lamotrigine. Side effects can be headache, eruption, fatigue, stupor, and impaired concentration.

Keppra is a fast growing drug. The share in the world market was 4.2 % in 2003.

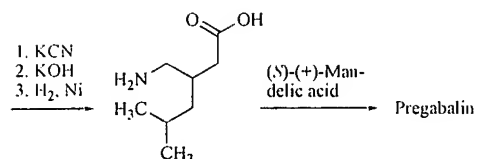
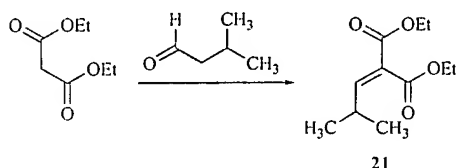
Pregabalin [148553-50-8], 3-(S)-3-(amino-methyl)-5-methylhexanoic acid, $\text{C}_8\text{H}_{17}\text{NO}_2$, M_r 159.23, mp 186 °C; white solid.



Trade name: Lyrica (Pfizer).

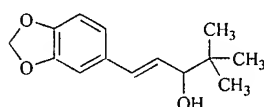
Synthesis: Diethyl malonate reacts with 3-methylbutanal by means of dipropylamine to 21. This intermediate is transformed to racemic pregabalin. The racemate is submitted to optical resolution with S-(+)-mandelic acid [67]. In Ref. [68] the resolution of racemic 3-isopropylglutaramic acid is described.

Pregabalin is also obtained by enantioselective syntheses [69].



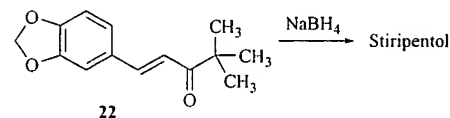
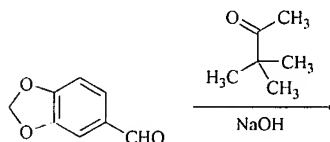
Pregabalin is a GABA analogue and blocks L-type calcium channels. The efficacy in terms of responder rate, the facility in an add-on therapy by its pharmacokinetic profile and the reduced potential for drug-drug interactions compared to other antiepileptics resulted in a sales growth of 2000 % in 2004-2005 [70]. Side effects can be stupor, somnolence and weight gain.

Stiripentol [049763-96-4], 1-(1,3-benzodioxol-5-yl)-4,4-dimethyl-1-penten-3-ol, $\text{C}_{14}\text{H}_{18}\text{O}_3$, M_r 234.29, mp 74 °C; white solid.



Trade name: Diacomit (Biocodex)

Synthesis: The condensation of 3,4-methylenedioxybenzaldehyde with 3,3-dimethyl-2-butanone by means of NaOH gives 22, which is reduced with NaBH_4 [71].



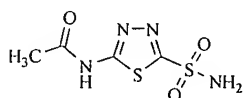
Stiripentol is a GABA aminotransferase inhibitor and a GABA reuptake inhibitor. Stiripentol is used as an add-on to clobazam and valproate in children with severe myoclonic epilepsy in infancy (Dravet's syndrome). Stiripentol is designated as an orphan medicine in EU. The most common side effects are anorexia, insomnia, drowsiness, and ataxia.

2.8. Other Antiepileptic Drugs

Sulfonamides. The sulfonamides acetazolamide and sulthiam have been known as anticonvulsants since 1952 and 1960, respectively. In most patients, the development of tolerance limits their usefulness as antiepileptic drugs. Their

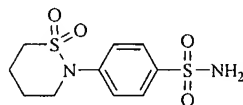
anticonvulsant action is due to the inhibition of carbonic anhydrase.

Acetazolamide [59-66-5], *N*-[5-(aminosulfonyl)-1,3,4-thiadiazol-2-yl]acetamide, 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide, $C_4H_6N_4O_3S_2$, M_r 222.25, (\rightarrow Diuretics, Chap. 2).



Trade name: Diamox (Lederle), Diamox (Sanofi-Avensis).

Sulthiam [61-56-3], 4-(tetrahydro-2*H*-1,2-thiazin-2-yl)benzenesulfonamide *S,S*-dioxide, $C_{10}H_{14}N_2O_4S_2$, M_r 290.37; (synthesis [4]).



Trade name: Ospolot (Desitin).

Bromides were first introduced as anti-epileptic drugs into therapy in 1857. Side effects and ongoing difficulties in defining a therapeutic range of bromide therapy made their use become obsolete. Now these drugs encounter a kind of comeback because of their good activity against tonic – clonic seizures in refractory patients.

Potassium bromide, KBr, **trade name:** Dibro-Be mono (Dibropharm).

3. References

1. D. Janz, "Antiepileptic Drugs," *Handb. Exp. Pharmacol.* **74** (1985) 3 – 34.
2. M. J. Eadie, "Antiepileptic Drugs," *Handb. Exp. Pharmacol.* **74** (1985) 765 – 790.
3. J. W. Sander, D. D. Shorvon, *J. Neurol. Neurosurg. Psychiatry* **50** (1987) 829 – 839.
4. T. Tomson, T. Walczak, M. Sillanpaa, J. W. Sander, *Epilepsia* **46** (2005) Suppl 11, 54–61.
5. J. M. Sutherland, H. Tait, M. J. Eadie (eds.): *The Epilepsies, Modern Diagnosis and Treatment*, 2nd ed., Churchill Livingstone, Edinburgh-London 1974.
6. M. A. Dichter, *Epilepsia* **30** (1989) Suppl. 1, 829 – 839.
7. M. A. Dichter, Overview: The Neurobiology of Epilepsy, in J. Engel Jr., T. A. Pedley (eds.): *Epilepsy: A Comprehensive Textbook*, Lippincott-Raven Publishers, Philadelphia New York 1997.
8. Commission on Classification and Terminology of the International League Against Epilepsy, *Epilepsia* **22** (1981) 489.
9. J. Engel Jr., *Epilepsia* **42** (2001) 796–803.
10. C. Biervert et al., *Science* **279** (1998) 403 – 406.
11. H. Lerche, N. Mitrovic, F. Lehmann-Horn, *Fortschr. Neurol. Psychiatr.* **65** (1997) 481 – 488.
12. D. Schmidt, in M. N. G. Dukes (ed.): *Meyler's Side Effects of Drugs*, 12th ed., Elsevier Science, Amsterdam 1992 122 – 143.
13. H. G. Wieser, *Baillieres-Clin-Neurol.* **5** (1996) 849 – 875.
14. K. D. Laxer, *West. J. Med.* **161** (1994) 309 – 314.
15. J. W. Wheless, R. E. Ramsay, S. D. Collins, *Neurotherapeutics* **4** (2007) 163–172.
16. J. M. Pellock, E. Faught, I. E. Leppik, S. Shinnar, M. L. Zupanc, *Epilepsy Res.* **71** (2006) 89–101.
17. C. Chiron, *Neurotherapeutics* **4** (2007) 123–127.
18. H. J. Kupferberg, *Pharmac. Weekbl. Science ed* **143A** (1992) 132 – 138.
19. W. Löscher, D. Schmidt, *Epilepsy Res.* **17** (1994) 95 – 134.
20. W. Löscher, D. Schmidt, *Epilepsy Res.* **2** (1988) 145 – 181.
21. W. Löscher, C. Rundfeldt, *J. Pharmacol. Exp. Ther.* **258** (1991) 483 – 489.
22. Smith M. et al., discovery of antiepileptic drugs. *Neurotherapeutics* **4**, 12 – 17. 2007
23. B. A. Roecklein, H. J. Sacks, H. Mortko, J. Stables, *Neurotherapeutics* **4** (2007) 97–101.
24. L. Almeida, P. Soares-Da-Silva, *Neurotherapeutics* **4** (2007) 88–96.
25. M. Bialer, B. Yagen, *Neurotherapeutics* **4** (2007) 130–137.
26. B. S. Meldrum, M. A. Rogawski, *Neurotherapeutics* **4** (2007) 18–61.
27. R. J. Porter, V. Nohria, C. Rundfeldt, *Neurotherapeutics* **4** (2007), 149–154.
28. I. M. Kapetanovic, C. Rundfeldt, *CNS Drug Rev.* **2** (1996) 308–321.
29. B. A. Lauria-Horner, *Exp. Opin. Invest. Drugs* **12** (2003) 663–672.
30. P. Rosenstiel, *Neurotherapeutics* **4** (2007) 84–87.

31. B. Bennett, A. Matagne, P. Michel, M. Leonard, M. Cornet, M. A. Meeus, N. Toublanc, *Neurotherapeutics* **4** (2007) 117–122.
32. W. J. Brouillette, G. B. Brown, T. M. DeLorey, G. Liang, *J. Pharm. Sci.* **79** (1990) 871–874.
33. V. Pestellini et al., *J. Med. Chem.* **23** (1988) 473–476.
34. A. Haj-Yehia, M. Bialer, *Pharmaceut. Res.* **6** (1989) 683–689.
35. J. Lange, W. Kazmierski, J. Daroszewski, *Pol. J. Pharmacol. Pharm.* **43** (1991) 71–77.
36. H. Schäfer, “Antiepileptic Drugs,” *Handb. Exp. Pharmacol.* **74** (1985) 199–243.
37. J. A. Bikker, J. Kubanek, D. F. Weaver, *Epilepsia* **35** (1994) 411–425.
38. G. Klopman, R. Contreras, *Mol. Pharmacol.* **27** (1985) 86–93.
39. K. Unverferth et al., *J. Med. Chem.* (1998) 63–73.
40. S. M. Tasso et al., *J. Mol. Model.* **2001**, 231–239.
41. R. H. Levy, R. H. Mattson, B. S. Meldrum (eds.): *Antiepileptic Drugs*, 4th ed., Raven Press, New York 1995.
42. M. Dam, L. Gram (eds.): *Comprehensive Epileptology* Raven Press, New York 1991.
43. E. Perucca, *Br. J. Clin. Pharmacol.* **42** (1996) 531–543.
44. A. G. Marson, Z. A. Kadir, J. L. Hutton, D. W. Chadwick, *Epilepsia* **38** (1997) 859–880. D. W. Chadwick, *Epilepsia* **39** (1998) 799–803.
45. R. S. Fischer, J. T. Coyle: Neurotransmitters and Epilepsy, in I. Bodis-Wollner, E. A. Zimmermann (eds.): *Frontiers of Clinical Neuroscience*, vol. **11**, Wiley-Liss, New York 1991.
46. Datamonitor, Commercial Insight: Anticonvulsants, Reference Code: DMHC2017, Publication Date: 09/2004.
47. H. Biltz, K. Seydel, *Ber. Dtsch. Chem. Ges.* **44** (1911) 1–413.
48. Parke-Davis, US 2 409 754, 1946 (H. R. Henc).
49. Geigy, US 2948718, 1960 (W. Schindler).
50. Geigy, DE 2 011 087, 1970 (W. Schindler).
51. Geigy, EP 28 028, 1979 (A. Aufderhaar).
52. O. Lohse, U. Beutler, P. Funfschilling, P. Furet, J. France, D. Kaufmann, W. Zaugg, *Tetrahedron Lett.* **2001**, 385–389.
53. B. S. Burton, *Am. Chem. J.* **3** (1882) 385–395. W. Keil, *Hoppe-Seyler's Z. Physiol. Chem.* **282** (1947) 137–142.
54. S. Sahay, G. Sircar, *J. Chem. Soc.* 1927, 1252–1256.
55. J. S. H. Davis, W. H. Hook, F. Long, *J. Chem. Soc.* 1950, 32–41.
56. ICI, GB 666 027, 1952 (W. R. Boon, H. C. Carrington, C. H. Vasey).
57. L. H. Sternbach, R. I. Fryer, O. Keller, W. Metlesics, G. Sach, N. Steiger, *J. Med. Chem.* **6** (1963) 261–265.
58. Merrell, BE 873 766, 1978 (M. W. Gittos, G. J. Leterte).
59. Wellcome, GB 7 919 257, 1979 (M. G. Baxter, A. R. Elphick, A. A. Miller, D. A. Sawyer).
60. Gödecke, DE 2 460 891, 1974 (G. Satzinger, J. Hartenstein, M. Herrmann, W. Heldt). DE 2 611 690, 1977 (J. Hartenstein, G. Satzinger).
61. B. E. Maryanoff et al., *J. Med. Chem.* **30** (1987) 880–887.
62. K. E. Anderson et al., *J. Med. Chem.* **36** (1993) 1716–1725.
63. B. J. Ludwig, L. S. Powell, F. M. Berger, *J. Med. Chem.* **12** (1969) 462–472.
64. Dainippon, US 4 172 896, 1979 (H. Uno, O. Takatsuki). H. Uno, M. Kurokawa, Y. Masuda, H. Nishimura, *J. Med. Chem.* **22** (1979) 180–183.
65. UCB SA, AU 8542530, 1984 (J. Gobert, J.-P. Geerts, G. Bodson).
66. UCB SA, GB 2225322, 1988 (E. Cossement, G. Motte, J.-P. Geerts, J. Gobert).
67. UCB SA, WO 2001062726, 2000 (E. Differding et al.).
68. Pfizer Inc., WO 1996040617, 1995 (T.M. Grote et al.).
69. Pfizer Inc., WO 1996038405, 1995 (D. M. Sobieray, B. K. Huckabee).
70. L. Martin, X. Rabasseda, P. Leeson, J. Castaner, *Drugs Fut.* **24** (8) (1999) 862–870.
71. M. F. O'Neill, R. A. McArthur, Innovations in CNS, Business Insights, Chap. 8, 2006.
72. US 3910959, 1975 (F. M. J. Vallet).
73. Bayer, DE 1 111 191, 1959 (B. Helferich, R. Behnisch, Wirth); US 2916489, 1959 (B. Helferich, R. Behnisch).

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Evaluation of Epileptic Dogs as an Animal Model of Human Epilepsy^{*)}

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Summary: In 126 epileptic dogs with spontaneously recurring generalized tonic-clonic (grand mal) seizures, epidemiological aspects and the efficacy of chronic oral treatment with common antiepileptic drugs were studied. Furthermore, the pharmacokinetics of antiepileptic drugs in dogs was compared with the values known for man. As in man, idiopathic epilepsy appeared to be more common than symptomatic epilepsy in dogs. There was a preponderance of male vs. female animals. When the breeds of the epileptic dogs were compared to the distribution of breeds in the hospital population, breed-related differences in the prevalence of epilepsy were found. The highest prevalence was seen in Cocker spaniels, Miniature schnauzers, Collies and Bassets. The total prevalence of dogs with epilepsy was 0.55%. Comparison of pharmacokinetics of antiepileptic drugs showed that some drugs were suited for maintenance therapy in dogs (primidone, phenobarbital, ethosuximide, trimethadione) whereas others appeared not to be ideally suited because of their short half-lives (phenytoin, carbamazepine, valproic acid, diazepam, clonazepam, nitrazepam). This was confirmed by the evaluation of antiepileptic drug efficacy in epileptic dogs. 46 dogs were treated with primidone at daily doses of 14–104 mg/kg for 6–60 months. During medication with primidone, effective plasma levels of its metabolite phenobarbital could be maintained. Complete control of seizures or a reduction of seizure frequency by at least 75% was achieved in 39% of the dogs at phenobarbital concentrations of 5–49 µg/ml. Similar figures were obtained during chronic treatment with phenobarbital at daily doses of 2.5–13 mg/kg. However, during chronic treatment with phenytoin, carbamazepine, and valproic acid it proved impossible to maintain effective plasma concentrations, even when high doses were administered 3 times daily. The data indicate that the epileptic dog is a suitable model of human epilepsy and offers a number of interesting epidemiological, pharmacokinetic and drug efficacy characteristics. The model provides special advantages for the study of efficacy of chronic antiepileptic drug treatment, provided the pharmacokinetics of the drug to be studied allows the maintenance of effective drug levels.

Zusammenfassung: Eignung des epileptischen Hundes als Tiermodell für die Epilepsie des Menschen

An 126 epileptischen Hunden mit wiederholt und spontan auftretenden generalisierten tonisch-klonischen (Grand mal) Anfällen wurden epidemiologische Aspekte sowie die Wirksamkeit von gebräuchlichen Antiepileptika bei oraler Dauerbehandlung untersucht. Ferner wurde die Pharmakokinetik von Antiepileptika beim Hund mit vom Menschen her bekannten Daten verglichen. Wie beim Menschen schien idiopathische Epilepsie beim Hund häufiger zu sein als symptomatische Epilepsie. Epilepsie trat bei Rüden häufiger auf als bei Hündinnen. Ein Vergleich der Rassen epileptischer Hunde mit der Rassenverteilung in der Klinikpopulation ergab Rassenunterschiede: die höchste Prävalenz fand sich beim Cocker-Spaniel, Zwergschnauzer, Collie und Basset. Zusammengekommen betrug die Prävalenz beim Hund 0,55%. Der Vergleich pharmakokinetischer Daten von Antiepileptika ließ einige Stoffe beim Hund für eine Dauerbehandlung geeignet erscheinen (Primidon, Phenobarbital, Ethosuximid, Trimethadion), während andere aufgrund einer sehr kurzen Halbwertszeit als weniger geeignet eingeschätzt wurden (Phenytoin, Carbamazepin, Valproinsäure, Diazepam, Clonazepam, Nitrazepam). Die Untersuchung der Wirksamkeit einiger Antiepileptika beim epileptischen Hund bestätigte diese Einschätzung. 46 Hunde wurden für 6–60 Monate mit Primidon behandelt, die Tagesdosen betrugen 14–104 mg/kg. Während der Behandlung wurden wirksame Konzentrationen des Metaboliten Phenobarbital im Plasma aufrechterhalten. Anfallsfreiheit oder Reduktion der Anfallsfrequenz um wenigstens 75% wurde bei 39% der Hunde erreicht, wobei Phenobarbital-Konzentrationen von 5–49 µg/ml bestimmt wurden. Ähnliche Ergebnisse wurden bei Dauerbehandlung mit Phenobarbital erzielt, die Tagesdosen betrugen 2,5–13 mg/kg. Bei chronischer Behandlung mit Phenytoin, Carbamazepin und Valproinsäure war es dagegen nicht möglich, effektive Plasmakonzentrationen aufrechtzuerhalten, obwohl hohe Dosen dreimal täglich verabreicht wurden. Die Ergebnisse lassen den epileptischen Hund als Modell für die Epilepsieforschung geeignet erscheinen. Besondere Vorteile ergeben sich für die Untersuchung der Wirksamkeit bei Dauerbehandlung mit Antiepileptika, vorausgesetzt, daß die Pharmakokinetik des zu untersuchenden Antiepileptikums das Aufrechterhalten wirksamer Konzentrationen ermöglicht.

Key words: Antiepileptic drugs, clinical efficacy, pharmacokinetics · Epilepsy, experimental

1. Introduction

Epilepsy in man is generally characterized by chronically recurring, spontaneous clinical seizures. Experimental re-

search on epilepsy and antiepileptic drugs has mostly been done in mice and rats, in which seizures were induced by chemical or electrical methods [1]. Though these rodent models have proved useful for the development of new antiepileptic drugs, they are obviously not closely related to human epilepsy. An ideal animal model for epilepsy should show the following characteristics:

1. the development of spontaneously occurring recurrent seizures,

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2. a type of seizure similar in its clinical phenomenology to seizures occurring in human epilepsy.
3. pharmacokinetics of antiepileptic drugs similar to those in man thus allowing the maintenance of effective drug levels during chronic treatment.
4. effective plasma concentrations of antiepileptic drugs similar to those reported for human epilepsy.

No model at present meets all these criteria.

Among domesticated animals, epilepsy seems to be fairly common in dogs [2, 3]. In search of an animal model that approximates human epilepsy more closely than the models presently used, we embarked on a systematic study of epileptic dogs.

2. Material and methods

2.1. Epileptic dogs

From a total of about 22,000 dogs observed at the Department of Small Animal Diseases from January 1, 1978 to December 31, 1982, 126 dogs were included in this study. Inclusion criteria were:

1. generalized tonic clonic seizures (grand mal) with loss of consciousness,
2. repeated and spontaneous occurrence of these seizures,
3. no clinical or laboratory evidence for extracerebral etiology of seizures, such as hypoxia due to cardiovascular or pulmonary disease, hypoglycemia, hypocalcemia, hepatopathies and nephropathies.

The etiology of the epilepsy was classified as symptomatic if there was any clinical or pathological evidence for central nervous system disease, e.g. brain tumors, head trauma, encephalitis, leptomeningitis and hydrocephalus. In 18 of 22 cases classified as symptomatic, dogs had or were suspected to have brain tumors. Idiopathic epilepsy was assumed in the absence of any clinical or pathological indication of symptomatic etiology.

2.2. Evaluation of antiepileptic drugs

Details of antiepileptic therapy in dogs with grand mal epilepsy are shown in Table 1. Mosts dogs were treated ambulatory.

A total of 46 dogs was used for evaluation of the antiepileptic efficacy of primidone. All dogs were treated for at least 6 months. At the onset of treatment, the daily dose of primidone was gradually increased to about 30 mg/kg of body weight, divided into 2 or 3 oral

doses. Primidone was given as commercially available tablets or as commercially available liquid formulation, the latter allowing a better adaptation of dose to body weight in small breeds. The dosage was further increased if the seizures were not controlled. If the seizures were controlled by the initial dose, it was sometimes cautiously reduced. The average duration of treatment with primidone in the dogs used for the prospective drug trial was 24 months (range: 6–60 months). Blood samples were taken at various intervals for determination of plasma concentrations of primidone and its metabolites phenobarbital and phenylethylmalonamide (PEMA). This was done by gas chromatography as described previously [4]. Blood samples were usually collected 3 h after the morning dose had been given. Previous studies had shown rather large variations in the plasma concentrations of primidone and PEMA during continued treatment with primidone in dogs, because both compounds have a very short half-life [5, 6]. In contrast, concentrations of phenobarbital accumulated in plasma reaching a plateau after 6–8 days of primidone treatment. In fact, from these studies phenobarbital was estimated to be responsible for about 85% of the antiepileptic effect of continued medication with primidone. Therefore, in the present study only phenobarbital plasma concentrations were used for the monitoring of primidone treatment.

In a number of dogs in which primidone was ineffective, other antiepileptic drugs (phenytoin, carbamazepine or valproic acid) were added to the treatment. During medication, plasma levels of these drugs were monitored by gas chromatography [4]. Details of treatment with phenytoin, carbamazepine and valproic acid are shown in Table 1. Phenytoin and carbamazepine were given as commercially available tablets and valproic acid was administered in the form of enteric coated tablets (also from commercial source).

Besides primidone, a second prospective trial is under way with phenobarbital. Up to now, 17 dogs have been treated with phenobarbital for at least 6 months (see Table 1). Phenobarbital was given as commercial tablets, and plasma concentrations of the drug were determined by gas chromatography [4].

2.3. Pharmacokinetic studies in non-epileptic dogs

The pharmacokinetics of various antiepileptic drugs have been determined in healthy dogs, mostly Mongrels (see Table 3). Details of the respective experiments have been published previously [5, 7–10, 17, 18, 20–24].

Table 1: Evaluation of antiepileptic drugs in dogs with grand mal epilepsy. 1 to the efficacy of treatment see 3.3.

Antiepileptic drug	Dose ^{a1} (mg/kg ¹ /d p.o.)	Duration of treatment ^{a1} (months)	Number of dogs	Sex		Age at onset of drug evaluation ^{a2} (years)	Breeds
				Male	Female		
Primidone	4 (0) (14–104)	24 (6–60)	46	32	14	4.8 (0.4–11.0)	10 Cocker spaniels, 7 Poodles, 8 Alsatians, 4 Collies, 2 Basets, 2 Miniature schnauzers, 2 Boxers, 1 Mongrel, 8 others
Phenytoin ^{b1}	4 (0) (23–57)	5.1 (2–8)	4	2	2	8 (4.6–13.1)	1 Poodle, 1 Boxer, 1 Beagle, 1 Yorkshire terrier
Carbamazepine ^{b1}	16 ^b (9–24)	2.6 (0.5–4)	7	3	4	5.5 (3–11.8)	2 Poodles, 2 Alsatians, 1 Cocker spaniel, 1 Collie, 1 Schnauzer
Valproic acid ^{b1} (sodium salt)	172 ^c (150–186)	1.4 (1.5–5)	3	1	2	6 (4–9.5)	1 Cocker spaniel, 1 Alsatian, 1 Boxer
Phenobarbital	1.2 ^d (2.5–13)	11 (7–28)	17	11	6	5.3 (1.5–11.5)	4 Alsatians, 2 Cocker spaniels, 2 Miniature schnauzers, 1 Collie, 1 Baset, 4 Mongrels, 3 others

^{a1} Mean and range. ^{a2} In addition to treatment with primidone. ^{b1} Divided into 2 doses. ^{b2} Divided into 2–3 doses. ^{c1} Divided into 3 doses.

Table 2: Distribution of breeds in dogs with grand mal epilepsy. Data refer to dogs which were examined at the Department of Small Animal Diseases, Berlin, from 1978 to 1982. For comparison, distribution of breeds is also shown for all dogs which were brought to the Department in these years.

Breed	No. of dogs with epilepsy				No. of all dogs		Percentage of epileptic dogs	
	Idiopathic		Symptomatic					
	Male	Female	Male	Female	Male	Female	Male	Female
Cocker spaniel	13	8	1		835	669	1.68	1.14
Alsatian	18	4	1	1	2,298	1420	0.70	0.28
Poodle	8	4		1	1,411	1,588	0.78	0.07
Miniature schnauzer	3	2			115	107	2.61	1.87
Collie	1	2			156	143	1.92	1.50
Baset	5	1			162	70	3.09	1.43
Other breeds	17	12	5	4	5,090	4,550	0.41	0.35
Mongrels	4	4	2	4	1,346	1,697	0.34	0.48
All	68	36	12	10	17,116	9,994	0.66	0.46

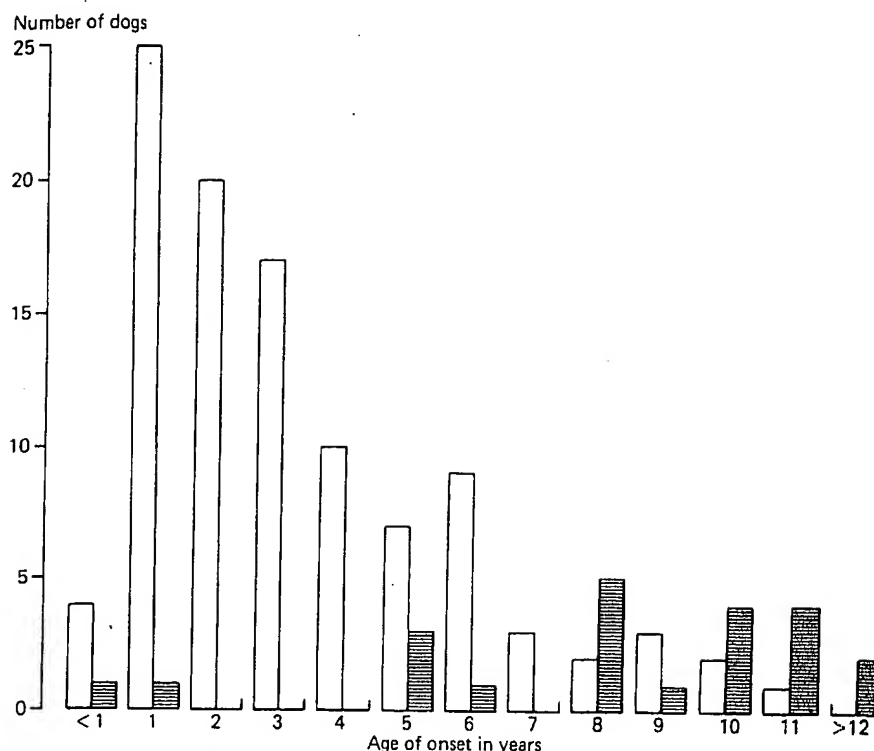


Fig. 1: Distribution of age of onset of grand mal epilepsy in 126 dogs. □ Idiopathic epilepsy; ▨ symptomatic epilepsy.

3. Results

3.1. Epidemiology of epilepsy in dogs

A detailed epidemiological study is in progress and will be reported later. Only general observations are reported here.

The epidemiological evaluation of 126 epileptic dogs showed a preponderance of male vs. female animals (Table 2). When the breeds of the epileptic dogs were compared to the distribution of breeds in the hospital population, breed-related differences in the prevalence of epilepsy were found (Table 2). The highest prevalence was seen in Cocker spaniels, Miniature schnauzers, Collies and Bassets. Epilepsy occurring in these breeds was, with one exception, classified as idiopathic. The total prevalence of dogs with epilepsy was 0.55%. However, the hospital population may not accurately reflect the population of dogs at large.

The age of onset of epilepsy was 4 years or lower in 62% of the animals. When the etiology of the epilepsy was consid-

ered, the age of onset was markedly higher in the group with symptomatic epilepsy (Fig. 1). Idiopathic epilepsy was found in the majority of dogs (Table 2, Fig. 1).

3.2. Pharmacokinetics of antiepileptic drugs in dogs

We have previously determined the pharmacokinetics of common antiepileptic drugs in non-epileptic dogs. In Table 3, the respective pharmacokinetic parameters are summarized and compared with the values known from man. The apparent volume of distribution and the plasma protein binding of most antiepileptic drugs are similar in dogs and man. Furthermore, the half-life of elimination in dogs is comparable to that in man for phenobarbital, primidone, ethosuximide and trimethadione. In fact, previous studies with the latter four drugs in (healthy) dogs have shown that it is possible to maintain effective plasma concentrations by continued oral treatment [5, 7, 8]. However, it should be

Table 3: Comparison of pharmacokinetic parameters of antiepileptic drugs in dog and man. Dog studies were performed in non-epileptic animals, mostly Mongrels. Data were taken from ref. [5, 7-10, 16-25].

Antiepileptic drugs and main active metabolites	Half-life (h)		Volume of distribution (l/kg)		Plasma protein binding (%)	
	Dog	Man	Dog	Man	Dog	Man
Phenobarbital	50-89 (25-38)*	70-100	0.6-0.7	0.5-1	46	50
Primidone	10-12 (4-6)*	6-12		0.6	19-23	19-24
Phenobarbital	as above	as above	as above	as above	77	90-93
Phenytoin	1.5-6	15-20	1.0	0.5-0.7	70	73-75
Carbamazepine	1.1-1.9	25-50		0.8	40	46
Carbamazepine-epoxide	1.6-3.1	8-15	0.2-0.4	0.15-0.4	60-80	85-95
Valproic acid	0.8-2.7	8-15	0.25		97	94.5
2-en-valproate	1.8	12.5	0.4-0.7	0.7	1-9	6-12
Ethosuximide	11-26	30-70			7-9	9-12
Trimethadione	8	16-20			6-28	6-22
Dimethadione	50-80	240			95	97
Diazepam	1-5	24-72	11	1-2	96	97
Desmethyldiazepam	1-5	50-120			82	80
Clonazepam	1-3	24-36	2.4	1.5-4.4		86
Nitrazepam	1.7	17-31		1.5-2.7		

* Data in brackets were determined in Beagle dogs.

noted that in Beagle dogs the half-lives of at least phenobarbital and primidone are much shorter than in other dogs (Table 3).

The half-lives of phenytoin, carbamazepine, valproic acid, diazepam, clonazepam and nitrazepam are markedly shorter in dogs than in man. This indicates that the latter drugs are not ideally suited for chronic antiepileptic drug trials in epileptic dogs. In fact, recent studies with phenytoin, carbamazepine and valproic acid in healthy dogs have demonstrated that even with high daily doses effective plasma concentrations cannot be maintained [9-11]. This is especially true for phenytoin and carbamazepine which during continued treatment in dogs display a strong reduction in half-life, due to induction of microsomal liver enzymes [9, 10]. As regards the bioavailability of antiepileptic drugs after oral administration, our previous studies showed that for most drugs evaluated comparable figures are obtained in dog and man [8, 10, 21, 22].

3.3. Prospective trial with antiepileptic drugs in dogs with epilepsy

Primidone was chosen as a first drug for a prospective trial in 46 epileptic dogs. The efficacy of drug therapy was evaluated in 3 categories (Table 4). As shown in Table 5, primidone was an effective antiepileptic agent in 18 out of 46 ani-

Table 4: Criteria for the efficacy of antiepileptic drug therapy in epileptic dogs. All dogs used for drug evaluation were treated for at least 6 months.

Group I	Complete control of generalized tonic clonic seizures for longer than four times the longest seizure interval in the previous course of epilepsy.
Group II	Reduction of generalized tonic clonic seizure frequency by 75% or more. The actual seizure frequency was compared during the first and the last six months of drug treatment. Some animals had additional types of epileptic seizures.
Group III	No change in generalized tonic clonic seizure frequency or a reduction of less than 75%.

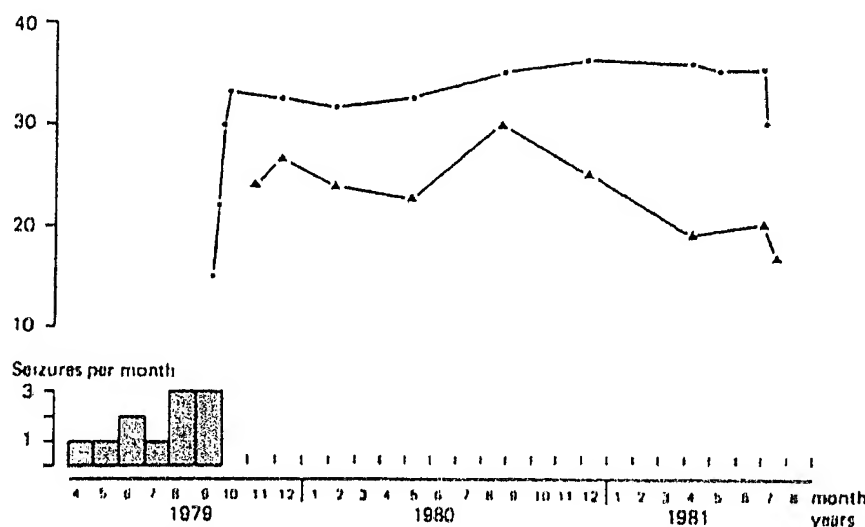


Fig. 2: Response to treatment with primidone of a dog with generalized tonic clonic seizures. Frequency of seizures is indicated by the number of seizures per month. According to the efficacy of drug therapy this dog was assigned to group I (see Table 4). ● — — — ● Daily dose of primidone (mg/kg); ▲ — — — ▲ phenobarbital plasma concentration (µg/ml).

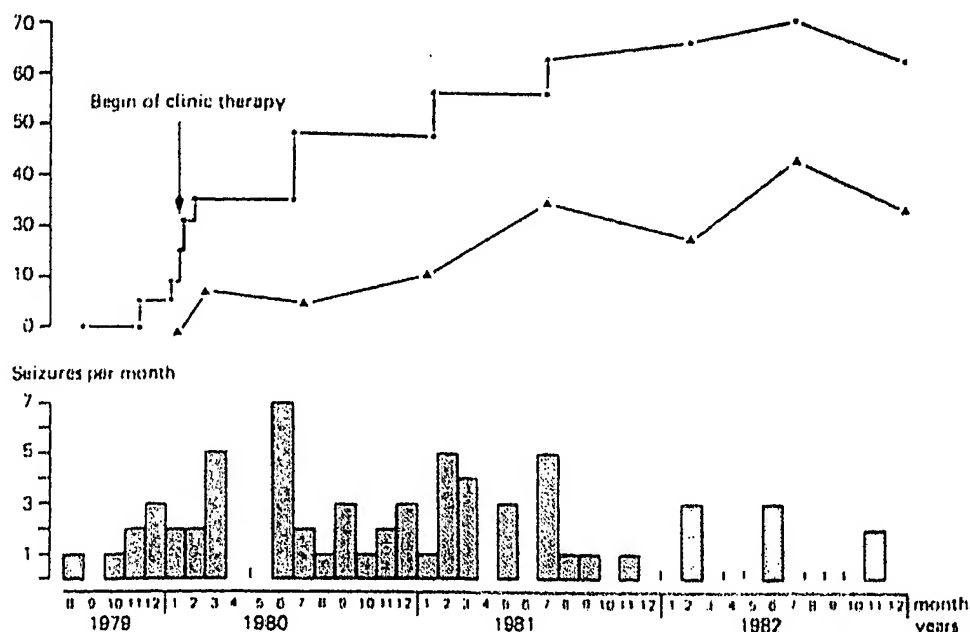


Fig. 3: Response to treatment with primidone of a dog with generalized tonic-clonic seizures. Frequency of seizures is indicated by the number of seizures per month. The arrow indicates the onset of therapy in the hospital, controlled by monitoring of phenobarbital plasma concentrations. According to the efficacy of drug therapy this dog was assigned to group II (see Table 4). ● — — — ● Daily dose of primidone (mg/kg); ▲ — — — ▲ phenobarbital plasma concentration (µg/ml).

Table 5: Results of primidone treatment in 46 epileptic dogs. For explanation of drug efficacy groups see Table 4.

Group	No. of dogs	Mean and range of daily dose of primidone (mg/kg)	Mean and range of plasma phenobarbital concentrations ($\mu\text{g/ml}$)
I	12 ^{a)}	32 (14–45)	23 (5–39) ^{b)}
II	6	49 (24–71)	30 (20–49) ^{b)}
III	28	47 (24–104)	32 (15–58) ^{c)}

a) Average duration of seizure control was 23 months (range: 9–49 months).
b) Lowest effective steady state plasma concentration.
c) Highest steady state plasma concentration.

mals. Twelve of these dogs became completely free from generalized tonic clonic seizures, but 2 dogs continued to have additional types of epileptic seizures. The effective plasma concentrations of phenobarbital ranged from 5–39 $\mu\text{g/ml}$ with a mean of 23 $\mu\text{g/ml}$ (for example see Fig. 2). In 6 dogs grand mal seizure frequency was reduced by at least 75% (for example see Fig. 3). Two of these dogs continued to have additional types of epileptic seizures.

According to the strict criteria used, primidone was not considered to be effective in 28 dogs. In some dogs of this group the efficacy of treatment might have been improved by a further increase in dosage. However, in 8 dogs of group III (with phenobarbital plasma concentrations of 23–58 $\mu\text{g/ml}$) a further increase of the daily dose was precluded by the development of side effects, which included sedation, weakness of the hind limbs, polydipsia, polyuria, and polyphagia. These side effects also appeared in most dogs of groups I and II shortly after the onset of treatment but usually disappeared in the course of chronic medication. In some dogs treated at high doses for longer periods, the values for glutamic pyruvic transaminase, glutamate dehydrogenase and alkaline phosphatase increased markedly.

Addition of either carbamazepine (9–24 $\text{mg kg}^{-1} \text{d}^{-1}$), phenytoin (23–57 $\text{mg kg}^{-1} \text{d}^{-1}$ or valproic acid (150–186 $\text{mg kg}^{-1} \text{d}^{-1}$) to primidone medication in dogs of group III resulted in no clear reduction of seizure frequency or severity. As could be expected from the pharmacokinetic studies in healthy dogs (see 3.2.), even with the high doses of these drugs used it proved impossible to maintain effective plasma concentrations during chronic treatment.

An initial evaluation of 17 dogs on monotherapy with phenobarbital (2.5–13 mg/kg/d) for 7–25 months indicates that phenobarbital is just as effective as primidone. In fact, complete control of seizures was obtained in 5 dogs, and 1 dog showed a reduction of seizure frequency by more than 75%. The effective plasma concentrations of phenobarbital ranged from 11–27 $\mu\text{g/ml}$. Side effects were similar to treatment with primidone, however, there were no comparable effects on liver enzymes.

4. Discussion

Previous reports of different Schools of Veterinary Medicine have indicated that epilepsy is a major disease of the central nervous system in dogs [2, 3, 12, 13]. In these studies, clinical and electroencephalographic observations suggested that epilepsy in dogs closely approximates the disease in man. Accordingly, the usefulness of epileptic dogs as an animal model of human epilepsy has repeatedly been emphasized [12, 13], yet there has been no systematic comparative evaluation of the efficacy of antiepileptic drugs in this model.

The present data indicate that the epileptic dog is indeed a suitable model of human epilepsy. As in man, idiopathic epilepsy appears to be more common than symptomatic epilepsy. Previous studies have strongly suggested that idiopathic epilepsy in dogs may be hereditary, at least in certain breeds [12, 13]. The present epidemiological data on breed distribution of grand mal seizures support this suggestion. Furthermore, the data demonstrate age and sex-related differences in epilepsy of dogs. The prevalence of epilepsy in dogs seems to be similar to that in man.

Generalized seizures of grand mal type are by far the most common type of seizures in dogs, but there are also other types of epileptic seizures common in dogs which is an additional advantage of this animal model. This aspect has to be studied further both clinically and by electroencephalographic observation. The present communication deals primarily with generalized tonic clonic seizures. These seizures occur spontaneously and are not dependent on sensory stimulation like seizures in other genetic animal models of epilepsy, such as the photosensitive baboon *Papio papio*, audiogenic seizure susceptible mice, and gerbils with reflex epilepsy.

A comparison of the pharmacokinetics of common antiepileptic drugs suggests similar characteristics in man and dogs for primidone, phenobarbital, ethosuximide and trimethadione. This is important as the epileptic dog is a model for chronic epilepsy in contrast to other acute seizure models, and thus pharmacokinetics of antiepileptic drugs should allow maintenance therapy. Consequently, the short half-lives of a number of other antiepileptic drugs in dogs limit the usefulness of the epileptic dog as a model for antiepileptic drug evaluation. In fact, the preliminary data on chronic treatment with valproic acid, phenytoin and carbamazepine indicate that these major antiepileptic drugs are not effective in the dog model.

The prospective trial of primidone in the epileptic dog model demonstrates that primidone is an effective antiepileptic agent in 39% of the animals. This corresponds well with the efficacy reported for primidone treatment in human epilepsy with generalized tonic clonic seizures [14]. Furthermore, the mean and range of the effective plasma concentrations of phenobarbital is in good agreement with the values reported in trials of epileptic patients [14]. Preliminary evaluation of a group of epileptic dogs treated with phenobarbital suggests that this drug has the same efficacy as primidone. It is of interest to note that 61% of the dogs did not show a significant reduction of seizure frequency ($\geq 75\%$) during primidone treatment. This figure seems to be similar for phenobarbital. Thus, the epileptic dog model may be suitable for the investigation of drug resistance in epilepsy which is a major difficulty in the management of the disease in man [15].

Unfortunately, there are some drawbacks of the epileptic dog model. These are

1. the differences in pharmacokinetics of some major antiepileptic drugs between dog and man, and
2. the logistical difficulties of obtaining an adequately high number of animals.

Nevertheless, the epileptic dog model offers a number of interesting epidemiological, pharmacokinetic and drug efficacy characteristics, and may provide special advantages for the study of seizure development, age-related drug effects and chronic drug treatment.

5. References

- [1] Purpura, D. P., Penry, J. K., Tower, D., Woodbury, D. M., Walter, R. (Eds.), *Experimental Models of Epilepsy*, Raven Press, New York (1972) – [2] Croft, P. G., *Vet. Rec.* 77, 438 (1965) – [3] Cunningham, J. G., *J. Amer. Vet. Med. Ass.* 158, 589 (1971) – [4] Löscher, W., Göbel, W., *Epilepsia* 19, 463 (1978) – [5] Frey, H.-H., Göbel, W., Löscher, W., *Arch. Int. Pharmacodyn. Ther.* 242, 14 (1979) – [6] Schwartz-Porsche, D., Trautvetter, E., Göbel, W., Kleintier-Prax. 26, 209 (1981) – [7] Frey, H.-H., Schulz, R., *Acta Pharmacol.* 28, 477 (1970) – [8] El-Sayed, M. A., Löscher, W., Frey, H.-H., *Arch. Int. Pharmacodyn. Ther.* 234, 180 (1978) – [9] Frey, H.-H., Löscher, W., *Arch. Int. Pharmacodyn. Ther.* 243, 180 (1980) – [10] Frey, H.-H., Löscher, W., *Amer. J. Vet. Res.* 41, 1635 (1980) – [11] Löscher, W., *J. vet. Pharmacol. Ther.* 4, 111 (1981) – [12] Holliday, T. A., Cunningham, J. G., Gutnick, M. J., *Epilepsia* 11, 281 (1970) – [13] Edmonds, H. L., Hegreberg, G. A., van Gelder, N. M., Sylvester, D. M., Clemons, R. M., Chathburn, C. G., *Fed. Proc.* 38, 2424 (1979) – [14] Schmidt, D., *Behandlung der Epilepsien*, Georg Thieme Verlag, Stuttgart (1981) – [15] Schmidt, D., *J. Neurol. Neurosurg. Psychiat.* 45, 1119 (1982) – [16] Bochner, F., Caruthers, G., Kampmann, J., Steiner, J., *Handbook of Clinical Pharmacology*, Little, Brown and Company, Boston (1978) – [17] Löscher, W., *J. Pharmacol. Exp. Ther.* 204, 255 (1978) – [18] Löscher, W., *J. Pharmacol. Exp. Ther.* 208, 429 (1979) – [19] Eadie,

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RESEARCH**

D-23129: a new anticonvulsant with a broad spectrum activity in animal models of epileptic seizures

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Abstract

The anticonvulsant activity of the novel drug D-23129 (*N*-(2-amino-4-(4-fluorobenzylamino)phenyl)carbamic acid ethyl ester) was evaluated in animal models of epileptic seizures. D-23129 was active after oral and intraperitoneal administration in rats and mice in a range of anticonvulsant tests at nontoxic doses. The compound was active against electrically induced seizures (MES, ED₅₀ rat p.o. = 2.87 mg/kg), against seizures induced chemically by pentylenetetrazole (s.c. PTZ, ED₅₀ mouse p.o. = 13.5 mg/kg), picrotoxin and *N*-methyl-D-aspartate (NMDA) and in a genetic animal model, the DBA/2 mouse. It was not active against seizures induced by bicuculline and strychnine. Motor impairment, evaluated with the rotarod test and by observation in the open field, was minimal at doses showing anticonvulsant activity. D-23129 was very effective in elevating the threshold for electrically and chemically induced seizures. Considering the dose increasing the MES threshold by 50% (TID₅₀ mouse i.p. = 1.6 mg/kg; TID₅₀ rat i.p. = 0.72 mg/kg) and the TD₅₀ obtained in the rotarod test, the protective index of D-23129 is better than that of valproate and phenytoin. During 14 days chronic oral treatment with 15 mg/kg, no development of tolerance was observed. D-23129 thus presents an orally active, safe, broad spectrum anticonvulsant agent, which is structurally unrelated to anticonvulsants currently used. We expect that D-23129 will improve the treatment of refractory seizures in humans.

Keywords: Complex partial seizure; Carbamazepine; Phenytoin; Phenobarbital; Valproate; Electroshock; Pentylenetetrazole; Seizure threshold

1. Introduction

The worldwide prevalence of epilepsy is estimated at between 0.3 and 0.6% [19,21]. About 20–

30% of patients suffer from intractable epilepsy or severe side effects despite the early treatment and an optimum daily dosage of an adequate antiepileptic drug [2,8,20].

A possible approach to develop drugs which are different from existing anticonvulsants, i.e. which

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might prove to be more effective in patients with drug-resistant epilepsy, is to seek chemical entities with broad spectrum anticonvulsant properties in many animal models of epilepsy, which are not related to drugs currently marketed.

Flupirtine is marketed as a centrally acting analgesic. In a serendipitous approach to anticonvulsant therapy the drug was submitted to the Antiepileptic Drug Development (ADD) program at NIH. Flupirtine was found to have potent anticonvulsant effects in many animal models of epilepsy and also in a pilot study in humans [22]. Molecular modelling studies of flupirtine and its analogues allowed us to define a pharmacophore for central analgesic activity composed of a phenyl ring and a basic nitrogen atom at a specified distance from this ring [23]. This pharmacophore enabled us to separate the analgesic from the anticonvulsant activity for this class of compounds. Thus, it was found that the absence of the basic nitrogen atom (in our case the pyridine nitrogen) enhances the antiepileptic activity while at the same time the analgesic activity is reduced. Furthermore, quantum mechanical calculations yielded very similar electron charge distributions in flupirtine and desazaflupirtine derivatives. Based on these observations a synthesis program was initiated which resulted in the development of a number of desazaflupirtine derivatives as a new class of anticonvulsants. The most potent was D-20443, the dihydrochloride of D-23129. These results were later confirmed by quantitative structure–activity relationship (QSAR) studies. Clinical trials with flupirtine were subsequently discontinued due to the development of these more potent analogues.

In the present study, the anticonvulsant activity of D-23129 (Fig. 1) was evaluated in a variety of electrical and chemical seizure models and in a genetic model of epilepsy, the DBA/2 mouse. Fur-

thermore, to check for development of tolerance during chronic treatment, a common problem of many drugs with anticonvulsant properties, D-23129 was also evaluated during 14 days chronic treatment in a sensitive and graded seizure model, the maximal electroshock seizure threshold test. Some of the data have been published in abstract form [17,30,31].

2. Materials and methods

2.1. Animals

Male mice (CrI: NMRI BR) were obtained at the age of 4 weeks (body weight 19–27 g) from Harlan Winkelmann (Borchen) for the acute threshold models and from Charles River (Sulzfeld) for all other experiments. They were allowed to adapt to the laboratory environment for one week before the experiments were started. All experiments with drug injections were then carried out within the next week to minimize the effect of increasing age on drug susceptibility. Each mouse was used for only one experiment.

Female Wistar rats (Harlan Winkelmann, Borchen, age of 11–12 weeks, body weight 180–200 g) were used for the MES threshold determination. All other experiments were performed with male Wistar rats which were obtained from Charles River (Sulzfeld) at a body weight of 120–150 g. They were used after at least one week of adaptation to the laboratory. Each rat was used for only one experiment.

Mice and rats were kept in groups of five in plastic cages at controlled temperature (25°C) and humidity (about 50–60%) with a 12-h light cycle beginning at 6 a.m. They received standard laboratory rodent chow and tap water ad libitum.

2.2. Methods

Experiments were performed in three different laboratories within the NIH Antiepileptic Drug Development Program (ADD) in the Anticonvulsant Screening Project: the Department of Pharmacology and Toxicology of the University of Utah in Salt Lake City, the Department of Pharmacology, Toxicology and Pharmacy of the School of Veterinary

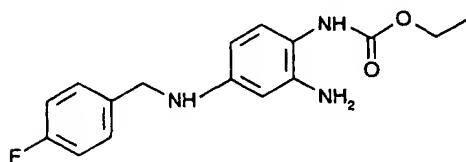


Fig. 1. Chemical structure of D-23129, *N*-(2-amino-4-(4-fluorobenzylamino)phenyl)carbamic acid ethyl ester. Molecular weight: 303.3 g/mol. Molecular formula: $C_{16}H_{18}FN_3O_2$.

Medicine in Hannover (Germany) and the Pharmacological Department of Arzneimittelwerk Dresden GmbH (AWD) in Radebeul (Germany). Experimental procedures in the MES and PTZ seizure tests were matched between NIH and AWD; all seizure threshold models were performed in Hannover.

2.2.1. Maximal electroshock seizure (MES) test, mice and rats

Mice and rats were stimulated with corneal electrodes, using a suprathreshold fixed current sinus wave stimulus (50 Hz, 50 mA, 0.2 s for mice and 50 Hz, 150 mA, 0.2 s for rats) using a stimulator with adjustable current strength which delivered a constant current adjustable from 1 to 300 mA regardless of the impedance of the test object. The maximal tonic extension of the hindlimbs was used as endpoint. In control groups (with vehicle injection) all animals exhibited tonic extension of hindlimbs. An approximation of the time of peak effect was achieved by administering D-23129 to groups of mice and rats at different pretreatment times and determining the percentage of animals protected. The time at which the highest protection was achieved was used for all seizure tests except the MES threshold and i.v. PTZ threshold tests. The determination of median effective dose (ED_{50}) in the MES test was conducted at this time with at least three groups of 8–10 mice and rats, respectively. Data obtained previously [33] with phenytoin, carbamazepine and valproate using the same procedure as data obtained with phenobarbital were included in the evaluation for comparison of anticonvulsant activity. The ED_{50} and 95% confidence intervals were calculated by probit analysis [7].

2.2.2. Maximal electroshock seizure threshold test, mice and rats

The threshold for seizures induced by maximal electroshock in mice and rats was determined via corneal electrodes by means of a constant-current stimulator with a sinus wave stimulus (50 Hz, 0.2 s). The stimulus intensity was varied by an up-and-down method in which the current was lowered or raised in 0.06 log intervals if the preceding animal did or did not show hindlimb extension, respectively. The data thus generated in groups of 20 mice or 15 rats for each dose tested were used to calculate the threshold

current for inducing hindlimb extension in 50% of the mice (CC_{50}), with confidence limits for 95% probability, using the method of Kimball et al. [6]. In acute experiments, each group of animals was used for only one threshold determination. Control groups, which received the vehicle used for drug administration, were tested together with the drug-treated animals, using the same pretreatment time at which the drug was tested. In case of significant drug effect (tested by Student's *t*-test), the dose increasing the MES threshold by 50% (TID_{50}) was determined by log-linear regression analysis from the dose-response curves, using at least three doses. For more details on MES seizure models, see Löscher et al. [11].

To determine the effect of subchronic treatment on anticonvulsant effect, 2 groups of 20 mice were treated as follows: Group 1—D-23129 15 mg/kg p.o. for 14 days, followed by a 6-day period of vehicle administration. Group 2—Vehicle p.o. for 20 days. The anticonvulsant activity was measured 15 min after administration on day 1, 3, 7, 14, 15, 16 and 20 using the MES threshold test with corneal stimulation. Such a procedure with repeated threshold determination in one group of animals is a good way to minimize interindividual variability in response to drug treatment. The repeated determination of the MES threshold via corneal electrodes is well tolerated by mice and does not influence the responsiveness of the animals to the drug treatment [18]. To abolish acute pain reactions possibly associated with repeated corneal stimulation, the local anaesthetic lidocaine (2% solution) was applied to the eyes 2–3 min before the stimulation. The significance of differences in the CC_{50} value between control and treated mice and between acute (day one) and chronic (day 3, 6 and 14) treatment was calculated using Student's *t*-test.

2.2.3. Chemically induced seizures

2.2.3.1. The threshold for the pentylenetetrazole (PTZ)-induced seizures.

Different seizure stages are elicited in male mice if PTZ is infused as a 1% solution into the tail vein at a rate of 0.3 ml/min with an infusion pump. In untreated control mice, the following seizure types occurred during PTZ infusion (in order of appearance): 1, one or more gener-

alized myoclonic twitches of the whole body; 2, repeated clonic seizures of fore and/or hind limbs without loss of righting reflexes; 3, a generalized clonus with repeated clonic seizures of fore- and hindlimbs, during which loss of their righting reflexes occurs; 4, clonic seizure with loss of righting reflexes followed by forelimb tonic extension; 5, tonic hindlimb extension (not always observed, animals sometimes die without showing tonic hindlimb extension). In the experiments with D-23129, the stages 1, 3 and 4 were used as endpoints. To determine the thresholds for the different endpoints, groups of 10–12 mice were used for each dose tested. The drug was administered intraperitoneally at the time of peak effect, 5 min for mice and 10 min for rats, determined in preceding experiments (Löscher and Hönack, unpublished data), before the start of infusion of PTZ solution. The thresholds for the three endpoints were calculated as the dose (mg/kg) of PTZ inducing the seizure stages during infusion. All drug-treated groups were compared with vehicle-treated groups on each day. The effect of the drug treatment was calculated for each endpoint separately as percent increase over control of PTZ dose needed to elicit the respective seizure stage. The significance of differences in PTZ dose between control and treated mice was calculated using Student's *t*-test. In case of significant drug effects, the dose increasing the PTZ threshold by 50% (TID₅₀) was determined by log-linear regression analysis from the dose–response curves, using at least three doses.

2.2.3.2. S.c. PTZ seizure test, mice. For the s.c. PTZ test, a dose–response curve of PTZ was determined using groups of 10 mice. PTZ was injected s.c. in the back of the neck of the animals. The animals were then observed for 30 min after injection and the first generalized clonic seizure with loss of righting reflexes was used as the endpoint. In this way, the dose inducing this seizure type in 97% of the mice (CD₉₇) was calculated in mice (85 mg/kg) by the method of Litchfield and Wilcoxon [7]. After administration of these doses, the following seizure stages were observed in untreated mice and rats (described in the order of appearance): 1, one or more generalized myoclonic twitches of the whole body; 2, repeated clonic seizures of fore- and/or hindlimbs for more than 3 s without loss of righting reflexes

(corresponding to the threshold seizure proposed by Swinyard [25] for anticonvulsant drug evaluation in the s.c. PTZ test); 3, a generalized clonic seizure of fore- and hindlimbs, during which animals exhibited loss of righting reflexes; 4, loss of righting followed by tonic forelimb seizure; 5, loss of righting with tonic fore- and hindlimb seizure. In the controls with or without vehicle injection, endpoints 1–3 were observed in 9–10 animals per group of 10 animals. The number of animals experiencing higher seizure scores varied. For drug potency studies, PTZ was injected s.c. in groups of 10 animals at the time of the maximal anticonvulsant effect and the animals were observed for 30 min for the occurrence of seizures. Animals not experiencing a 3 s clonic episode (stage 2) or higher seizure scores within the 30 min observation period were considered as protected. Vehicle treatment did not affect PTZ-induced seizures. ED₅₀ and 95% confidence intervals were calculated by probit analysis [7]. Data obtained previously [33] with phenytoin, carbamazepine and valproate using the same procedure were included in the evaluation for comparison of anticonvulsant activity. For more details on PTZ seizure models, see Löscher et al. [12].

2.2.3.3. S.c. picrotoxin, s.c. bicuculline and s.c. strychnine, mice. As with PTZ, the chemoconvulsants bicuculline, picrotoxin and strychnine were administered subcutaneously to mice in a dose eliciting convulsions in 97% of control mice. The doses needed were 2.7, 2.5 and 1.2 mg/kg, respectively. With bicuculline and picrotoxin, a suppression of a 3 s clonic episode (stage 2 as described with PTZ) was used as the endpoint. With strychnine, animals not experiencing any tonic seizure components were counted as being protected. Observation time with all convulsants was 30 min. ED₅₀ and 95% confidence intervals were calculated by probit analysis [7]. Data obtained previously [33] with phenytoin, carbamazepine and valproate using the same procedures were included in the evaluation for comparison of anticonvulsant activity.

2.2.3.4. I.c.v. NMDA seizure tests, mice. NMDA, if applied via the i.c.v. route, elicits typical seizures depending on the dose applied [24,27]. At low doses, mice experience intense episodes of clonic seizures.

If the dose of NMDA is raised approximately 10-fold, tonic extension of forelimbs is the predominant seizure type elicited in mice. The dose needed to elicit the clonic seizures and tonic forelimb extension in 97% of mice was determined to be 0.2 $\mu\text{g}/5 \mu\text{l}$ and 3 $\mu\text{g}/5 \mu\text{l}$, respectively, using the method described previously [24,27]. For anticonvulsant drug potency experiments, the anticonvulsant was administered in at least three doses via the i.p. route to groups of 8–10 mice and at the time of peak effect (determined in the MES test), NMDA was administered. The animals were then observed for the occurrence of seizure signs for 30 min. ED_{50} and 95% confidence intervals were calculated by probit analysis [7].

2.2.4. Genetic epilepsy model: the audiogenic seizure susceptible mouse

Male DBA/2J audiogenic-seizure-susceptible mice (21–22 days of age, 6–10 g body weight, maximal susceptibility to sound-induced seizures) from the Bomholtgard Breeding and Research Centre Ltd., Ry, Denmark were used. The mice were placed in a circular plastic cage (19 cm diameter) and were exposed to a sound stimulus of 110 dB (12 kHz) for 60 s, while being observed for the occurrence of seizures. The severity of seizures was ranked as follows [32]: 0—no response, 1—wild running for < 10 s, 2—wild running for > 10 s, 3—clonic seizure, 4—forelimb extension/hindlimb flexion, 5—tonic seizure, 6—respiratory arrest. The test was carried out 15 min after i.p. or oral drug administration. Significant differences between same day control and drug-treated animals were calculated using the Mann–Whitney *U*-test. The ED_{50} and 95% confidence intervals against audiogenic clonic (score < 2) seizures were calculated by probit analysis [7].

2.2.5. Motor impairment, mice and rats

Motor impairment, often termed as neurotoxicity, was identified in mice and rats by the rotarod procedure. Inability of an animal to maintain its equilibrium for one min in at least one of three trials on a rotating rod (6 rpm, diameter 2.5 cm for mice and 8 rpm, 6 cm diameter for rats) was used as an indication of such impairment. In rats, motor deficit (ataxia, abnormal gait and stance, loss of placing response and muscle tone) was also visually determined in an

open field. Untreated mice were able to remain on the rotating rod for several minutes, whereas rats had to be trained before drug experiments. TD_{50} and 95% confidence intervals were calculated by probit analysis [7].

2.2.6. Drugs

Phenytoin, carbamazepine, sodium valproate, phenobarbital, pentylenetetrazole, bicuculline, picrotoxin, strychnine and *N*-methyl-D-aspartate (NMDA) were obtained from commercial suppliers. D-23129 and D-20443, the hydrochloride of D-23129, were synthesized at ASTA Medica, Frankfurt [3]. Valproate, D-20443 and all chemoconvulsants were dissolved in 0.9% sodium chloride solution; phenytoin and carbamazepine were suspended in 0.5% methylcellulose in water, D-23129 was suspended in 0.5% hydroxyethylcellulose in water. The drugs and chemoconvulsants were administered in a volume of 10 ml/kg in mice and 4 ml/kg in rats with the exception of PTZ, which was administered s.c. in a volume of 2 ml/kg in rats. For the experiments on the MES threshold and i.v. PTZ thresholds, D-23129 was dissolved by means of 100 μl 1 M HCl and further dissolved with 0.9% NaCl solution to achieve the desired concentration. In these experiments, the drug was applied at a volume of 10 ml/kg and 3 ml/kg i.p. in mice and rats, respectively.

3. Results

3.1. Electrically induced seizures

As shown in Table 1, D-23129 was effective in the traditional MES test with suprathreshold stimulation. In preceding experiments, the time of peak effect in the MES seizure test was determined to be 15 min after both i.p. and p.o. administration in mice and 10 min after i.p. and 30 min after p.o. administration in rats; the time of peak effect for motor impairment was determined to be 5 min after i.p. and 15 min after p.o. administration in mice and 10 and 120 min after i.p. and p.o. administration in rats, respectively. In the MES test, the drug was effective in both mice and rats (ED_{50} D-23129 9.3 (6.3–13.1) mg/kg i.p. in mice, ED_{50} D-20443 (the hydrochloride of D-23129) 5.1 (3.8–6.8) mg/kg i.p. in rats).

Table 1
Anticonvulsant and neurotoxic activity of D-23129 and standard anticonvulsants in the MES seizure model and in the rotarod test in mice and rats

Substance	Effective doses in mice			Effective doses in rats						
	Time of test (min)	ED ₅₀ i.p. (mg/kg)	ED ₅₀ p.o. (mg/kg)	TD ₅₀ i.p. (mg/kg)	TD ₅₀ p.o. (mg/kg)	Time of test (min)	ED ₅₀ i.p. (mg/kg)	ED ₅₀ p.o. (mg/kg)	TD ₅₀ i.p. (mg/kg)	TD ₅₀ p.o. (mg/kg)
D-23129	15/15/5/15	9.3 ^b (6.3–13.1)	26.8 ^c (16.3–38.1)	20.5 (18.5–22.9)	63.4 ^c (51.4–84.6)	10/30/10/120	5.1 ^d (3.8–6.8)	2.87 ^b (1.93–4.14)	9.96 (2.9–34.0)	83.5 ^b (60.6–115)
Phenytoin	120/240/30/240	6.48 ^a (5.65–7.24)	8.59 ^a (7.19–9.69)	42.8 ^a (36.4–47.5)	88.6 ^a (80.4–98.5)	120	n.d.	23.2 ^a	n.d.	> 500 ^a (21.4–25.4)
Carbamazepine	15	9.85 ^a (8.77–10.7)	11.9 ^a (8.47–15.0)	47.8 ^a (39.2–59.2)	95.6 ^a (85.7–109)	60	n.d.	3.57 ^a (2.41–4.72)	n.d.	361 ^a (319–402)
Valproate	15 (237–359)	287 ^a (489–659)	571 ^a (412–571)	483 ^a	> 1000 ^a	30	n.d. (332–441)	395 ^a	n.d. (719–1148)	859 ^a
Phenobarbital	30	23.8 (19.9–28.5)	n.d.	76.5 (69.5–84.2)	n.d.	60	21.7 (19.2–24.5)	n.d.	56.6 (48.1–66.6)	

The tests were performed at the time of peak effect which was determined separately in the MES and rotarod test. With phenytoin, the MES in mice was performed 2 h after i.p. and 4 h after p.o. administration; the rotarod test was performed 30 min after i.p. and 4 h after p.o. administration. With D-23129, the anticonvulsant and neurotoxic effects in rats were determined 30 min and 120 min after p.o. administration; both measures were taken 10 min after i.p. administration. Data are given as ED₅₀ and 95% confidence interval calculated from at least three doses tested using probit regression [7]. N.d., not determined.

^a Data taken from White et al. for comparison [33].

^b Experiments were performed within the ADD program at NIH, Bethesda with D-23129.

^c D-20443 (the hydrochloride of D-23129) was used instead of D-23129 at NIH.

^d D-20443 was used instead of D-23129.

Table 2
Anticonvulsant activity of D-23129 in comparison to standard anticonvulsants in chemical seizure models in mice

Substance	Chemoconvulsant ED ₅₀ (mg/kg) and 95% confidence interval					
	s.c. PTZ	s.c. Pic	s.c. Bic	s.c. Strych	icv NMDA Clonus	icv NMDA forelimb tonus
D-23129	13.5 ^b (9.2–17.9)	18.6 ^c (14.1–26.2)	> 30 ^c	> 30 ^c	9.08 ^c (5.19–13.6)	4.00 ^c (2.61–5.78)
Phenytoin	> 50 ^a	> 60 ^a	> 60 ^a	> 60 ^a	8.59 ^a (6.64–14.1)	0.60 ^a (0.34–0.95)
Carbamazepine	> 50 ^a	28.9 ^a (23.9–41.6)	> 60 ^a	> 60 ^a	14.4 ^a (10.6–20.1)	3.01 ^a (2.60–3.77)
Valproate	209 ^a (176–249)	311 ^a (203–438)	437 ^a (369–563)	345 ^a (308–393)	146 ^a (121–162)	82.8 ^a (57.5–113)

The chemoconvulsants were administered at the time of peak effect (determined in the MES seizure model), i.e. for D-23129, carbamazepine and valproate 15 min and for phenytoin 120 min after i.p. administration. Bic, bicuculline; Pic, picrotoxin; Strych, strychnine; NMDA, *N*-methyl-D-aspartate. All chemoconvulsants except NMDA were administered via the subcutaneous route. Data are given as ED₅₀ calculated from at least three doses tested and 95% confidence interval using probability regression[7].

^a Data taken from White et al. for comparison [33].

^b Experiments were performed at NIH using standard anticonvulsants and D-23129.

^c Experiments were performed at NIH using the hydrochloride salt of D-23129, termed D-20443.

Table 3
Effect of D-23129 on the threshold for maximal (tonic hindlimb extension) electroshock seizures and on motor behaviour in mice

Drug	Mice				
	Dose (mg/kg i.p.)	Threshold (CC ₅₀ in mA)	TID ₅₀ (mg/kg i.p.)	TD ₅₀ rotarod (mg/kg i.p.)	P.I. TID ₅₀ /TD ₅₀
D-23129	0	11.8 (11.0–12.6)			
	1	16.0 (14.8–16.9) *			
	2.5	18.6 (15.0–23.1) *			
	5	22.9 (20.3–25.8) *			
D-23129			1.6	20.5 (18.5–22.9)	12.8
Phenobarbital			4.0 ^a	80 (75–86) ^a	20.0 ^a
Carbamazepine			1.5 ^a	33 (26–40) ^a	22.0 ^a
Phenytoin			5.4 ^a	50 (44–57) ^a	9.3 ^a
Valproate			69 ^a	430 (391–473) ^a	6.2 ^a
Ethosuximide			n.e. ^a	503 (459–556) ^a	n.a. ^a
Diazepam			2.7 ^a	4.7 (3.8–5.7) ^a	1.7 ^a
Clonazepam			0.65 ^a	1.2 (0.87–1.5) ^a	1.8 ^a

The seizure threshold (stimulation via corneal electrodes) was determined as CC₅₀ (with confidence limits of 95% probability) in groups of 20 mice. For D-23129, the effect of three different doses on the threshold is shown. Control groups received i.p. vehicle injection. Significant differences between controls and drug-treated groups are indicated (*: $P < 0.001$). The dose which increased the threshold by 50% (TID₅₀) was calculated from the dose–response curves for D-23129 [10,11]. The potency to impair the motor behaviour of D-23129 was determined using the rotarod procedure after i.p. administration. The TD₅₀ values (with confidence limits for 95% probability) were calculated from data determined in at least three groups of animals at the time of peak effect.

^a For comparison, TID₅₀ values for standard antiepileptic drugs previously determined in the same model in mice are shown (data taken from Löscher and Nolting [10]). N.e., not effective; n.a., not applicable.

Comparing the effective doses after p.o. and i.p. administration, the ratio is 2.9 in mice and 0.6 in rats indicating intestinal absorption in both species. The protective index calculated as TD_{50} rotarod/ ED_{50} MES was calculated to be 2.2 and 2.0 in mice and rats after i.p. administration, respectively. The value is better than for valproate (1.7, mice i.p.), but does not reach the protective index of phenytoin, carbamazepine and phenobarbital (6.6, 4.8 and 3.2, mice i.p.).

3.2. Chemically induced seizures

D-23129 also exerted pronounced effects against chemically induced seizures in mice including pentylenetetrazole, the GABA antagonist picrotoxin and the glutamate agonist selective for the *N*-methyl-D-aspartate receptor, NMDA. No activity could be observed against bicuculline- and strychnine-induced seizures. The effective doses in both the GABA-related (pentylenetetrazole and picrotoxin) and glutamate-related (NMDA) seizure models were within the same range as for protection against electrically induced seizures in the MES test. A similar spectrum of activity was observed only for valproate. (Table 2,

data of standard anticonvulsants taken from White et al. [33] for comparison).

3.3. Threshold models

To determine the minimal dose of D-23129 capable of exerting anticonvulsant activity, threshold variations of the MES and the PTZ seizure test were utilized. To assure a good absorption and good activity, the tests were performed at the time of peak effect after i.p. administration of a solution of D-23129, which proved to be 5 min in mice and 10 min in rats. D-23129 was highly capable of elevating the threshold for tonic hindlimb extension in mice and rats leading to a significant increase already after administration of 1 and 0.5 mg/kg, respectively. The dose capable of elevating the threshold by 50% (TID_{50}) was calculated by log-linear regression from the dose–response curve as 1.6 mg/kg in mice and 0.72 mg/kg in rats. The protective index calculated from the TID_{50} and the TD_{50} was 12.8 in mice and 13.8 in rats (see Table 3).

D-23129 was also capable of increasing the thresholds for the different seizure types elicited by i.v. administration of PTZ (Table 4). While fairly high doses were needed (TID_{50} 18.2 mg/kg) to

Table 4
Effect of D-23129 on the threshold for different seizure types induced by i.v. infusion of pentylenetetrazole (PTZ) in mice

Dose (mg/kg)	PTZ threshold (mg/kg)		
	Myoclonus	Clonus	Forelimb tonus
Control 1	33.3 ± 5.9	38.3 ± 7	72.6 ± 30.7
5	32.9 ± 4.6	55.7 ± 18.5 **	78.9 ± 9.1
10	36.6 ± 6.5	102 ± 19.1 ***	154 ± 60.2 ***
Control 2	34.4 ± 4.6	40.9 ± 7.5	86.6 ± 11.7
7.5	38.3 ± 4.9 *	72.6 ± 20.9 ***	> 133 ± 49.7 **
Control 3	33.2 ± 4.0	39.3 ± 4.8	75.1 ± 9.0
20	46.4 ± 13.8 ***	94.1 ± 33.4 ***	> 163 ± 69.8 ***
30	63.2 ± 28.5 ***	140 ± 21.4 ***	> 212 ± 12 ***
TID_{50}	18.2	5.0	7.0

The seizure threshold was calculated as the dose of PTZ (\pm S.D.) inducing the respective seizure types in all animals of a group of 10 mice. The effect of five different doses of D-23129 after 5 min after i.p. administration on the threshold is shown. Since the experiments were performed on three days, three control groups ($n = 10$) receiving vehicle were needed to compare the data generated with same-day controls. Significant differences between controls and drug-treated groups are indicated (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$, Student's *t*-test). The doses which increased the different thresholds by 50% (TID_{50}) were calculated from the log-linear dose–response curve [10,12].

increase the threshold for the first myoclonic twitch, the drug was much more effective in increasing the threshold for induction of the first generalized clonus and the forelimb tonus yielding to TID_{50} values of 5.0 and 7.0 mg/kg, respectively.

3.4. Genetic epilepsy model

In a genetic epilepsy model, the DBA/2 mouse, D-23129 was capable of significantly suppressing the sound-induced seizures at doses as low as 2 mg/kg i.p. and p.o. (U-test). The ED_{50} for suppression of clonic seizures was determined to be 2.3 (1.8–3.0) and 4.6 (3.0–7.2) mg/kg 15 min after i.p. (Fig. 2) and oral (data not shown) administration, respectively.

3.5. Subchronic anticonvulsant activity

To determine if tolerance against the anticonvulsant effect occurs, the MES threshold test was utilized as a sensitive and graded measure of anticonvulsant and, after withdrawal of the drug, possible pro-convulsant withdrawal associated effects of D-23129. After oral administration of 15 mg/kg for 14 days, the effect observed on-day one was preserved

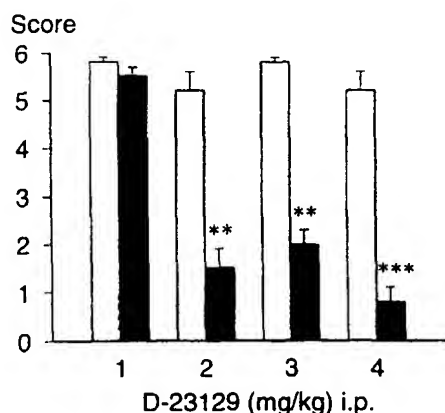


Fig. 2. Anticonvulsant activity of D-23129 against sound-induced seizures in male DBA/2J mice. The mice were placed in a circular plastic cage and were exposed to a sound stimulus of 110 dB (12 kHz) for 60 s, while being observed for the occurrence of seizures. The test was carried out 15 min after i.p. drug administration. **: $P < 0.01$; ***: $P < 0.001$, Mann-Whitney U -test. Open bars: vehicle control experiments; dashed bars: drug treatment.

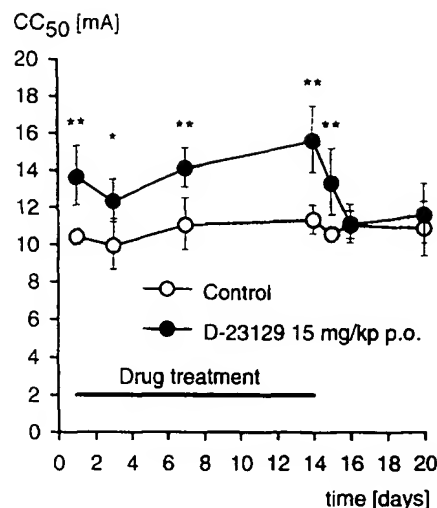


Fig. 3. Anticonvulsant activity of D-23129 in the MES threshold test during chronic daily treatment of 15 mg/kg p.o. in mice. The threshold to elicit tonic extension of the hind limb in 50% of the animals (CC_{50}) was repeatedly determined in two groups of 20 mice on day 1, 3, 7, and 14, one hour after p.o. administration of 15 mg/kg D-23129 (filled circles) or vehicle (open circles) in the morning. On day 15, 16 and 20, i.e. 24 h, 2 days and 6 days after termination of treatment, the threshold was determined in both groups 1 h after administration of vehicle. *: $P < 0.05$; **: $P < 0.01$; significant difference to respective control values, Student's t -test.

over the whole treatment time (Fig. 3). Furthermore, no rebound hyperexcitability was found. Instead, a small residual effect was present 24 hours after the last drug administration and was no longer present the next day.

3.6. Motor impairment

Mice were observed in the open field after i.p. administration of 10, 20, 30 and 40 mg/kg i.p. before rotarod testing. While no behavioural changes were observed after 10 mg/kg, mice showed flat body posture and some muscle relaxation as being observed by abdominal palpation after 20 mg/kg. Some animals were sensitive to noise and handling, exhibiting light twitches which occurred sometimes also spontaneously. After 30 mg/kg, some animals and after 40 mg/kg nearly all animals lost righting reflexes within 5–10 min for 2–3 min; 30 min after administration, side effects could no longer be observed. The TD_{50} on the rotarod was determined 5

min after i.p. administration to be 20.5 mg/kg (see Table 1). Rats were observed in the open field after administration of 5, 7.5, and 10 mg/kg i.p.. After 5 mg/kg, slight muscle relaxation, straub tail and some hypersensitivity as described for mice was present in some animals. After 7.5 mg/kg, flat body posture, muscle relaxation and straub tail and hyperexcitability was present in all rats. After 10 mg/kg the hyperexcitability was more pronounced. Rats showed an increased muscle tone in the limbs combined with a flat body posture. Upon handling, vocalization occurred in some animals. 45 min after administration, no side effects could be observed. The TD_{50} on the rotarod was determined 10 min after i.p. administration to be 9.96 mg/kg (see Table 1).

4. Discussion

D-23129 is a derivative of flupirtine which shows activity against both electrically and chemically induced seizures and which has exhibited some clinical efficacy in the treatment of drug-resistant patients with epilepsy (see Seaman et al. for review [22]). The clinical evaluation of flupirtine as an anticonvulsant was terminated due to the development of more potent analogues emerging from a synthesis line based on molecular modelling and quantitative structure–activity relationships [23]. These studies lead to the selection of D-23129 and its hydrochloride, D-20443, for further development. The activity in animal models of epileptic seizures of D-20443 proved to be substantially better than with flupirtine; taking the effective doses for the MES test in rats (ED_{50} for flupirtine in rats after oral administration: 47.3 mg/kg [22]), the ratio was bigger than 10.

First results regarding the anticonvulsant activity of D-20443 were published in 1993 by Nickel and co-workers in abstract form [13,14] indicating not only effectivity in the MES and s.c. PTZ test, but also against amygdala- and cornea-kindled seizures and penicillin-induced epileptic activity. The decision was made to develop the free base D-23129 instead of the dihydrochloride D-20443 because of technological reasons and a superior impurity profile. In our laboratory, D-23129 proved to be equipotent to D-20443 in the MES and s.c. PTZ models used

[17,30,31]. Observing mice and rats in the open field, D-23129 exerted some muscle relaxation already in the same dose range of anticonvulsant activity in the MES and PTZ seizure test. Muscle relaxation is also described for flupirtine [4] indicating that this effect might be a property of the chemical group. To rule out that the anticonvulsant effects of D-23129 are secondary to muscle relaxation, variations of the MES and PTZ test were utilized which are based on determination of threshold effects. Such a threshold approach was originally proposed by Swinyard and colleagues for anticonvulsant drug evaluation [26]. They calculated the dose capable of elevating the threshold 20% above control (threshold-increasing dose 20%, TID_{20}). Using this measure or the TID_{50} , the plasma concentrations of standard anticonvulsants (resulting from the lower doses needed) more closely resemble the plasma concentrations reached in humans during treatment [11,12,9]. In both the MES threshold and the i.v. PTZ threshold test, a very good activity could be demonstrated for D-23129 resulting in a clear cut separation of 'neurotoxic' motor impairment and anticonvulsant activity. The protective index (P.I.) calculated from the TID_{50} in the MES and the TD_{50} in the rotarod test is 12.8 for mice and 13.8 for rats, respectively. The value in mice is in the same range as the ratio for phenytoin, inferior to that of phenobarbital and carbamazepine, and is even better than the values for valproate, diazepam or clonazepam (Table 3).

Besides being highly active against electrically induced seizures, D-23129 also possesses activity against chemically induced seizures. Using the first generalized clonus as endpoint for the i.v. PTZ threshold test, the protective index calculated from the TD_{50} in the rotarod and the TID_{50} for first generalized clonus in the i.v. PTZ test for D-23129 is 4.1, also indicating a good separation in this respect. While D-23129 is also effective against picrotoxin-induced seizures in mice, it fails to show activity against bicuculline-induced convulsions. The reason for the different effect against direct GABA receptor antagonistic (bicuculline) versus GABA modulatory (PTZ, picrotoxin) convulsants remains unclear. Since D-23129 does not bind to the $GABA_A$ receptor (receptor affinity studies performed at Nova Screen, Kronbach, personal communication), it might be possible, that the drug, while positively modulating

the GABAergic system, needs direct GABAergic activity to do so. D-23129 did not only show activity against chemoconvulsants interfering with the inhibitory system, but also against convulsions induced with the glutamatergic agonist NMDA, again indicating a broad activity.

In our experiments, D-23129 has proven to be very active (ED_{50} 2.3 mg/kg i.p.) in a genetic model of epilepsy, the DBA/2 mouse [31]. Dailey et al. [1] obtained in two other genetic epilepsy models, the GEPR-3 and GEPR-9 rat, similar effective doses. The ED_{50} was calculated to be 1.56 mg/kg for the GEPR-3 and 6.24 mg/kg for the GEPR-9.

Yonekawa et al. [34] demonstrated that D-23129, besides being active in many *in vivo* models of epilepsy, also possesses some unique properties in an *in vitro* model of epileptic seizures, the hippocampal slice. In their study hyperexcitability and spontaneous bursting of the slice was induced by the potassium channel blocker 4-aminopyridine. D-23129 was capable of fully suppressing the spontaneous bursts in CA1 and CA3 areas. It also eliminated the afterdischarge-like trains of population spikes induced by a single electrical stimulation pulse without interfering with the normal evoked potential. These results demonstrate the unique activity of the drug, since none of the tested standards was capable of doing so without interfering with the normal evoked potential.

One of the major concerns with new anticonvulsant drugs is the development of tolerance and dependence. Nickel et al. [13,14] already reported that anticonvulsant activity of D-20443 in the MES test was preserved if the ED_{50} dose obtained in this test was administered over 5 or 7 days. We utilized a more subtle measure to find small changes in anticonvulsant activity. In one group of mice, the MES threshold test was repetitively performed during 14 days oral treatment of 15 mg/kg and for 6 days after termination. This method was proposed by Rundfeldt et al. [18] as a sensitive measure to evaluate the time course of tolerance development. Drugs like diazepam develop tolerance within three days to one week in this procedure. For D-23129, no reduction in threshold increase was observed. Furthermore, after termination of treatment, no withdrawal hyperexcitability was present. Instead, 24 hours after termination of treatment, a significant increase in thresh-

old was still present. Such a residual effect could be due to a long half life. While preliminary kinetic data based on plasma concentration of D-23129 during chronic treatment do not indicate any residual drug 24 h after the last administration, preliminary data obtained in brain homogenate indicate that the half life is longer and the concentration is higher in brain than in plasma (Jainta, personal communication). The results indicate that D-23129 has no pronounced potential to develop tolerance. However, it should be demonstrated that this holds true for longer treatment periods.

D-23129 has a broad spectrum anticonvulsant activity in many animal models of epileptic seizures including models possessing some predictability for generalized tonic clonic seizures like the MES, the DBA/2 mouse and the GEPR-9 rat and absence seizures like the s.c. PTZ model and the GEPR-3 rat. Furthermore, the drug has proven to be very active in the amygdala kindling model of focal epilepsy, which is considered to be the most predictive model for complex partial seizures [17,28,29]. While the spectrum of D-23129 is as broad as that of valproate, its potency is at least one magnitude better. To further evaluate the potential of the drug, it is mandatory to gain insight into the mode of anticonvulsant action. First results in this regard have been published by our group in abstract form [16,30]. D-23129 blocks voltage-dependent sodium and calcium channels, albeit at high concentrations [30]. Although D-23129 shows no affinity to the GABA receptor (receptor affinity studies performed at Nova Screen, Kronbach, personal communication), it influences the biological activity of GABA. Using standard electrophysiological techniques, it was demonstrated that D-23129 potentiates GABA-induced currents in cortical neurons in a dose-dependent manner at concentrations below $0.1 \mu\text{mol/l}$ [16]. A plausible explanation for the influence of D-23129 on the biological activity of GABA was given by means of molecular modelling studies [15]. Further insight in possible mechanisms of anticonvulsant action can be gained from the work of Kapetanovic et al. [5]. They demonstrated that D-23129 increased the amount of newly synthesized GABA in hippocampal slices. This effect was proposed to be due to a direct effect on cell metabolism, since it was not dependent on neuronal activity; addition of tetrodotoxin did not

abolish the increase in GABA synthesis. Taking the evidence on different mechanisms together, it becomes clear that D-23129 may not be comparable with conventional antiepileptics. Comparing the in vivo results with the proposed mechanisms of action, it is still possible that other mechanisms are involved, since the drug already shows pronounced anticonvulsant activity in the amygdala kindling model at very low doses. Such new mechanisms might be advantageous in that currently resistant patients could be eventually treated, but it might also yield currently unknown side effects. Hints for an unusual side effect spectrum can be taken from the open field experiments indicating hyperexcitability in combination with sedation and muscle relaxation at higher concentrations.

In conclusion, D-23129 has proven to be a broad spectrum anticonvulsant with oral activity in many animal models of epileptic seizures. Additional work is under way to further evaluate the drug in models of complex partial seizures and to clarify the mode of anticonvulsant action. Due to its unique chemical structure, the multiplicity of mechanisms of action and its good safety margin it is expected that D-23129 will provide an improved treatment against refractory epilepsy in the future.

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References

- [1] Dailey, J.W., Cheong, J.H., Ko, K.H., Adams-Curtis, L.E. and Jobe, P.C., Anticonvulsant properties of D-20443 in genetically epilepsy-prone rats: prediction of clinical response, *Neurosci. Lett.*, 195 (1995) 77–80.
- [2] Dam, M., Intractable epilepsy: Introduction. In: D. Schmidt and P.L. Morselli (Eds.), *Intractable Epilepsy: Experimental and Clinical Aspects*, Raven Press, New York, NY, 1986, pp. 1–3.
- [3] Dieter, H.-R., Engel, J., Kutscher, B., Polymeropoulos, E.E., Szelenyi, S. and Nickel, B., Pharmaceutically active 1,2,4-tri-amino-benzene derivatives, processes of their preparation and pharmaceutical compositions containing them, United States Patent No. US0005,384,330, 1995.
- [4] Jakovlev, V., Achterrath-Tuckermann, U., von Schlichtegroll, A., Strohm, F. and Thieme, K., Allgemeine pharmakologische Untersuchungen mit dem Analgetikum Flupirtin (general pharmacological studies with the analgesic flupirtine), *Arzneim.-Forsch./Drug Res.*, 35 (1985) 44–55.
- [5] Kapetanovic, I.M., Yonekawa, W.D. and Kupferberg, H.J., The effects of anticonvulsant compounds on 4-aminopyridine-induced de novo synthesis of neurotransmitter amino acids in rat hippocampus in vitro, *Epilepsy Res.*, 20 (1995) 113–120.
- [6] Kimball, A.W., Burnett, W.T. and Doherty, D.G., Chemical protection against ionizing radiation. I. Sampling methods for screening compounds in radiation protection studies with mice, *Radiat. Res.*, 7 (1957) 1–12.
- [7] Litchfield, J.T. and Wilcoxon, F., A simplified method of evaluating dose–effect experiments, *J. Pharmacol. Exp. Ther.*, 96 (1949) 99–113.
- [8] Loiseau, P., Do we need novel anti-epileptic drugs? *Br. J. Clin. Pract.*, 42 (1988) 2–3.
- [9] Löscher, W., von Hodenberg, A., Nolting, B., Fassbender, C.-P. and Taylor, C., Ralitoline: a reevaluation of anticonvulsant profile and determination of 'active' plasma concentrations in comparison with prototype antiepileptic drugs in mice, *Epilepsia*, 32 (1991) 560–568.
- [10] Löscher, W. and Nolting, B., The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. IV. Protective indices, *Epilepsy Res.*, 9 (1991) 1–10.
- [11] Löscher, W., Fassbender, C.P. and Nolting, B., The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models, *Epilepsy Res.*, 8 (1991) 79–94.
- [12] Löscher, W., Hönack, D., Fassbender, C.P. and Nolting, B., The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylentetrazol seizure models, *Epilepsy Res.*, 8 (1991) 171–189.
- [13] Nickel, B., Shandra, A., Godlevsky, L., Mazarati, A., Kupferberg, H. and Szelenyi, I., Antiepileptic effects of a new drug: D-20443, *Epilepsia*, 34 (Suppl. 2) (1993) 95.
- [14] Nickel, B., Shandra, A., Godlevsky, L., Mazarati, A., Kupferberg, H. and Szelenyi, I., Anticonvulsant activity of D-20443, *Naunyn-Schmiedenberg's Arch. Pharmacol.*, 347 (Suppl.) (1993) R142.
- [15] Polymeropoulos, E.E. and Kutscher, B., GABAergic activity of triamino-pyridines and triamino-benzenes. In: C.G. Wer-muth (Ed.), *Trends in QSAR and Molecular Modelling* 92, ESCOM, Leiden, 1993, pp. 456–460.
- [16] Rundfeldt, C., Rohlf, A. and Netzer, R., Multiple actions of the new anticonvulsant D-23129 on voltage gated inward currents and GABA-induced currents in cultured neuronal cells, *Naunyn Schmiedenberg's Arch. Pharmacol.*, 351 (Suppl.) (1995) R160.

- [17] Rundfeldt, C., Tober, C., Rostock, A. and Bartsch, R., The phenylcarbamic acid ester D-23129 elevates potently the threshold for amygdala kindled seizures in rats at nontoxic doses, *Epilepsie-Blätter*, 7 (1994) 29.
- [18] Rundfeldt, C., Wlaz, P., Hönack, D. and Löscher, W., Anticonvulsant tolerance and withdrawal characteristics of benzodiazepine receptor ligands in different seizure models in mice. Comparison of diazepam, bretazenil and abecarnil, *J. Pharmacol. Exp. Ther.* (in press).
- [19] Sander, J.W.A.S. and Shorvon, S.D., Incidence and prevalence studies in epilepsy and their methodological problems: a review, *J. Neurol. Neurosurg. Psychiat.*, 50 (1987) 829–839.
- [20] Schmidt, D., Anticonvulsants. In: M.N.G. Duke (Ed.), *Meyler's Side Effects of Drugs*, 12th edition, Elsevier, Amsterdam, 1992, pp. 122–143.
- [21] Schmidt, D. and Morselli, P.L. (Eds.), *Intractable Epilepsy: Experimental and Clinical Aspects*, Raven Press, New York, NY, 1986.
- [22] Seaman, C.A., Sheridan, P.H., Engel J., Molliere, M., Narang, P.K. and Nice, F.J., Flupirtine. In: B.S. Meldrum and R.J. Porter (Eds.), *New Anticonvulsant Drugs*, Libbey, London, 1986, pp. 135–146.
- [23] Seydel, J.K., Schaper, K.-J., Coates, E.A., Cordes, H.P., Emig, P., Engel, J., Kutscher, B. and Polymeropoulos, E.E., Synthesis and quantitative structure–activity relationship of anticonvulsant 2,3,6-triaminopyridines, *J. Med. Chem.*, 37 (1994) 3016–3022.
- [24] Singh, N.A., Swinyard, E.E. and White, H.S., Effects of prototype anticonvulsants on *N*-methyl-D-aspartate (NMDA)-induced seizures in mice, *Fed. Am. Soc. Exp. Biol. J.*, 2 (1988) A1068.
- [25] Swinyard, E.A., Laboratory evaluation of antiepileptic drugs. Review of laboratory methods, *Epilepsia*, 10 (1969) 107–119.
- [26] Swinyard, E.A., Brown, W.C. and Goodman, L.S., Comparative assay of antiepileptic drugs in mice and rats, *J. Pharmacol. Exp. Ther.*, 106 (1952) 319–330.
- [27] Swinyard, E.A., Wolf, H.H., White, H.S., Skeen, G.A., Stark, L.G., Albertson, T., Pong, S.F. and Drust, E.G., Characterization of the anticonvulsant properties of F-721, *Epilepsy Res.*, 15 (1993) 35–45.
- [28] Tober, C., Jainta, H., Rundfeldt, C., Rostock, A. and Bartsch, R., Anticonvulsant and antiepileptogenic effects of D-23129 in the kindling model of focal epilepsy, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 351 (Suppl.) (1995) R160.
- [29] Tober, C., Rostock, A., Rundfeldt, C. and Bartsch, R., D-23129: a potent anticonvulsant in the amygdala kindling model of complex partial seizures, *Eur. J. Pharmacol.* (in press).
- [30] Tober, C., Rundfeldt, C., Rostock, A. and Bartsch, R., The phenylcarbamic acid ester D-23129 is highly effective in epilepsy models for generalised and focal seizures at nontoxic doses, *Soc. Neurosci. Abstr.*, 20 (1994) 1641.
- [31] Tober, C., Rostock, A. and Bartsch, R., The phenylcarbamic acid ester D-23129 is highly effective against amygdala kindled seizures in rats, *Eur. J. Neurosci.*, (Suppl. 7) (1994) 212.
- [32] White, H.S., Patel, S. and Meldrum, B.S., Anticonvulsant profile of MDL 27,266: an orally active, broad spectrum anticonvulsant agent, *Epilepsy Res.*, 12 (1992) 217–226.
- [33] White, H.S., Wolf, H.H., Swinyard, A., Skeen, G.A. and Sofia, R.D., A neuropharmacological evaluation of felbamate as a novel anticonvulsant, *Epilepsia*, 33 (1992) 564–572.
- [34] Yonekawa, W.D., Kapetanovic, I.M. and Kupferberg, H.J., The effects of anticonvulsant agents on 4-aminopyridine induced epileptiform activity in rat hippocampus in vitro, *Epilepsy Res.*, 20 (1995) 137–150.

M. J., Tyrer, J. H., Anticonvulsant Therapy, Pharmacological Basis and Practice, 2nd ed. Churchill Livingstone, Edinburgh-London-New York (1980) - [20] Eliander, H., Pekkarinen, A., Acta Pharmacol. 47, 171 (1980) - [21] Löscher, W., Frey, H.-H., Arch. Int. Pharmacodyn. Ther. 254, 180 (1981) - [22] Löscher, W., Esenwein, H., Arzneim.-Forsch./Drug Res. 28 (I), 782 (1978) - [23] Löscher, W., Nau, H., J. Pharmacol. Exp. Ther. 226, 845 (1983) - [24] Altahan, E., Löscher, W., Frey, H.-H., Arch. Int. Pharmacodyn. Ther. 268, 180 (1984) - [25] Wülfelt, W., Nau, H., Rating, D., Helge, H., in: Stable Isotopes, Eds. H.-L. Schmidt, H. Förstel, K. Heinzinger, p. 265, Elsevier, Amsterdam (1982)

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Beeinflussung des oxidativen Stoffwechsels von Hirn-, Leber- und Herzmuskelhomogenaten durch Peptidderivate und Kälberblutdialysat in vitro

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Zusammenfassung: Untersucht wurden drei, aus unterschiedlichen Proteinen hergestellte Peptidderivate und ein durch Dialyse von Kälberblut gewonnener, eiweißfreier Extrakt. Für die einzelnen Präparationen wurden typische Effekte auf den oxidativen Stoffwechsel nachgewiesen. Eine vergleichbar hohe Stimulierung des Sauerstoffverbrauchs von Hirnhomogenaten, und zwar um 117–165%, wurde nach Applikation der Peptidderivate gemessen. Das peptidlangereicherte Derivat zeigte als einziges Testpräparat eine nicht organspezifische Wirkung und stimuliert den O_2 -Verbrauch von Gehirn, Leber und Herz um 165, 203 bzw. 202%, wobei die anderen untersuchten Präparate in ihrer Wirkung übertroffen wurden. Das Kälberblutdialysat, das die maximale Stimulierung mit 98% am Leberhomogenat zeigte, verursachte im Vergleich mit den Peptidderivaten, am Hirnhomogenat mit 51% eine eher geringe Stoffwechselsteigerung.

Summary: *In vitro* Effects of Peptide Derivatives and Extracts from Calf Blood on the Oxidative Metabolism of Brain, Liver and Heart Muscle Homogenates of the Rat

Three peptid preparations, derived from different proteins, and one protein free extract, produced by dialysis of calf blood, were tested. Typical effects of the individual preparations on oxidative metabolism could be shown. A comparable high stimulation of oxygen consumption of brain homogenates by 117 to 165% was measured after application of the peptide derivatives. One of them, the peptide enriched derivative, caused a high and not organ specific stimulation, increasing the oxygen consumption of brain, liver and heart muscle by 165, 203 and 202%, respectively, thus exceeding the effects of the other tested preparations. Extracts of calf blood, which showed maximal stimulation in liver homogenate (98%), led to rather small effects in brain homogenate (51%) compared with those induced by the peptide derivatives.

Key words: Cerebrolysin® · Peptide derivatives, *in vitro* effects, pharmacology · Protein free extracts · Psychoactive drugs